Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 9

Projects with a primary purpose of: Basic Research – Multisystemic and Other

Project Titles and keywords

1. **Development of novel PET and SPECT imaging biomarkers**
    - Imaging, PET, SPECT, radiotracer, biomarker

2. **Applying genetic engineering to the chicken**
    - Chicken, genetic engineering, development, health

3. **Analysis of plant ion channel and receptors**
    - SNAREs / K^+ channels / cell growth / proliferation

4. **Phenotyping Genetically Altered Mice**
    - Phenotyping, Ageing, Genetics, High-Throughput

5. **Spatial and temporal regulation of DNA replication**
    - DNA replication, cell proliferation, chromosome, nucleus

6. **Functional analysis of vertebrate enhancers**
    - Gene regulation, enhancers, conserved sequences, development

7. **Advancing methods for rederiving and archiving genetically altered mice**
    - Mouse, freezing, sperm, embryos, technology

8. **Molecular genomic and phenotypic analysis of environmental adaptations in Cyprinid fish**
    - Molecular genomic, phenotypic analysis, environmental adaptations, Cyprinid fish

9. **The European Xenopus Resource Centre**
    - European, Xenopus, Resource, Centre

10. **Early growth and life-long health**
    - Development, growth, genetics, epigenetics, metabolism

11. **Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Cardiovascular and Metabolic Disorders Diseases and Their Treatment**
    - NADPH oxidase; oxidative stress; cardiovascular diseases; insulin resistance; diabetes

12. **Effects of contaminants on fish physiology**
    - Fish, metals, contaminants, toxicology, physiology

13. **INVESTIGATING THE (PATHO)PHYSIOLOGICAL IMPORTANCE OF S-ACYLATION**
    - S-acylation, palmitoylation, zDHHC enzymes

14. **Improving the Pharmacokinetics and Tissue Targeting of Antimicrobials**
    - antimicrobial, melioidosis, Burkholderia pseudomallei, Francisella tularensis, Pharmacokinetics

15. **Molecular Regulation of Mammalian Development**
    - Genes , Oocyte, Development

16. **Developmental Dynamics of Tissue Formation**
    - Developmental biology, spinal cord, central nervous system

17. **Role of centrosomes, centrioles and cilia in vertebrate development**
    - Centrioles, cilia, vertebrate development

18. **Understanding gene function by phenotyping genetically altered mice**

19. **Production and Maintenance of Genetically Altered Mice**
    - Production, Geneticall Altered, Archiving

20. **Caveolae and endothelial transcytosis**
    - Caveolae, endothelium, blood vessel, endocytosis

21. **Deciphering the mechanisms of early embryonic development**
    - Vertebrate development, stem cells, xenopus

22. **Fungal infection, diagnosis and therapy**
    - Fungus, infection, antifungal, therapy, diagnosis

23. **Supply of Biospecimens from Rats and Mice**
    - Blood, Tissues, Drug discovery/development

24. **Preclinical imaging in Biomedical Research**
    - PET, imaging, drug

25. **Understanding mechanisms of fibrosis**
    - Fibrosis, scar, myofibroblasts, therapy, diagnosis

Project 1	**Development of novel PET and SPECT imaging biomarkers**	
Key Words (max. 5 words)	Imaging, PET, SPECT, radiotracer, biomarker	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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)	Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) imaging studies can help scientists and clinicians to understand more about the normal functions in the animal and human body, as well as, to learn how these functions change in different diseases. PET and SPECT imaging can also be used to evaluate the treatment results with new medicines. To obtain PET and SPECT images of a living organism, it is necessary to inject a substance called radiotracer. The PET or SPECT machine is then able to take images of the distribution of the radiotracer in the body. A radiotracer is a molecule that has been specifically designed so that it binds to a target inside the body. Currently there is a need to develop more radiotracers, so that scientists can study more functions in the body and develop better disease treatments. Also many radiotracers already available target only one clinical application, but previous research projects have shown examples where a single radiotracer can have many clinical applications. Consequently, there is a need to better explore the use of the already available radiotracers and expand their application to other clinical areas. This project aims to discover new PET and SPECT radiotracers for use in the clinic and in studies with new medicines. In addition, it aims to explore and expand the use of already available radiotracers to other research and clinical areas.	

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 discovery of new PET and SPECT radiotracers is worthwhile because there is a clinical need to better understand what causes animal and human diseases. New PET and SPECT radiotracers can also be used to detect and measure the amount of medicine that reaches the targeted area of the body. As a result, PET and SPECT imaging with radiotracers are often used as “imaging biomarkers”, i.e. techniques that can provide a measurable marker of biological processes in a living organism. Given that an intact body environment is complex and minimally accessible, imaging techniques such as PET and SPECT, that do not disturb the organism under investigation, are essential to increase our knowledge of how a given organ or system is affected in disease and how medicines act on it. Additionally, PET and SPECT imaging biomarkers could help governing bodies to issue guidelines on patient care and to decide on the utility of new medicines based on the imaging results. Finally, PET and SPECT imaging allows for detection of changes in the normal body earlier than other methods of diagnosis. Detecting disease early will have direct benefits on patients’ health and life quality. It will also shorten patient’s disability and recovery periods by reducing the need for invasive procedures and long rehabilitation processes. This will result in a reduction of the cost of disease management in the healthcare system and the whole society, because patients can return to their everyday live quicker.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate we will use 4300 rats and 2800 mice over the five year term of this PPL
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?	Potential adverse effects due to chronic tissue irradiation by repeated administration of a radiotracer and exposure to x-rays (e.g. several imaging sessions over time), pharmacological drugs administration (e.g. changes in animal temperature), repeat anaesthesia (e.g. changes in animal weight) and blood sampling (e.g. reduction of blood volume). These adverse effects are expected to be rare. Measures to minimize the occurrence of these effects will be implemented in all protocols (e.g. use of sophisticated blood sampling equipment to minimize risk of blood loss). All animals will be routinely and clinically evaluated for any signs of adverse effects throughout the work protocols. Whenever possible, measures to revert the adverse effects will be applied throughout the duration of each

	protocol (e.g. medicine antidotes, increase of animal temperature via heated mat, supplemental/soft foods to encourage eating). At the end of the protocols, animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The development of new PET and SPECT biomarkers rely on the evaluation of the radiotracer performance in a living organism. Because the living organism environment is complex, various functions are interconnected and contribute to the radiotracer distribution in the target tissues, it is not possible to recreate this with other non-animal alternatives. For example, blood circulation is essential for radiotracer delivery to the target. Additionally, the degradation and elimination of a radiotracer by a living organism is a dynamic and unique process that can't accurately be measured in other types of studies. The properties of each imaging biomarker will be studied using a multi-step approach. Only imaging biomarkers with optimal performance in non-animal experiments will progress into animal research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Small animal PET and SPECT machines will be used for imaging rats and mice. The use of this type of equipment substantially reduces the number of animals used (up to 80-90% depending on the study) compared with, for example, dissection and sampling techniques.
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 animal species to be used in this project will be rats and mice. The small animal PET and SPECT machines are best suited to image rats and mice. Additionally, the delivery of the radiotracer to the target site by blood circulation is central to PET and SPECT imaging, and rodents have a similar circulatory system to humans. Rats have larger size and blood volume compared with mice and, thus, are better suited for long imaging sessions (up to 6 hours) with serial blood collection over the imaging study. Mice are more amenable to genetic alterations compared with rats, thus providing a good platform for the development of animal models of human diseases. To minimise animal suffering, the duration of the experiments will be kept at the minimum required to reliably collect data from the experiment. The imaging studies will be conducted under general anaesthesia, and the radiotracers or any substances will be administered via the least invasive route and the smallest possible volume.

Project 2	**Applying genetic engineering to the chicken**	
Key Words (max. 5 words)	Chicken, genetic engineering, development, health	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to improve existing technologies for genetic engineering (GE) of the chicken, to introduce new advances if they are predicted to improve efficiencies and/or facilitate additional applications of GE tools. The current and improved technologies will be used to: (1) increase our understanding of how a chicken embryo develops during incubation of the egg. There are many processes in common between the development of the chicken embryo and the human embryo. The knowledge gained from studying chicken embryos will be very useful for understanding human development and diseases in which it is perturbed. (2) to increase our knowledge of the physiology of chickens, particularly in relation to the characteristics that are important for meat and egg production; (3) to understand in more depth the mechanisms involved in the response of chickens to infectious diseases, to aid development of more effective approaches to control of disease, including improved vaccines and genetic approaches; (4) to develop novel applications in biotechnology, including the utilisation of GE hens for production of therapeutic proteins in their eggs, for development of novel therapeutics for human and veterinary medicine; (5) develop novel approaches to poultry breeding by utilising GE to introduce novel	

	characteristics (e.g. resistance to avian influenza) and to introduce novel, beneficial gene variants to complement traditional poultry breeding and extend the beneficial genotypes of production chickens.
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 chicken is the most abundant livestock species (an estimated ~50-60 billion chickens hatched per year worldwide) and it is predicted that chicken meat will become the predominant source of meat in the human diet in the next few years. To meet this increasing demand, particularly in a sustainable way with consideration of animal welfare, we need to increase our knowledge of many fundamental aspects of avian biology. New knowledge gained using GE chickens will inform development of more effective vaccines. Increased understanding of genetic resistance and resilience to disease challenges using GE technologies will inform breeding strategies and may be applied directly to genetic improvement. Resistance to avian influenza (bird flu) infection has the potential to protect poultry from this continuing threat and to reduce the chances of transmission of bird flu to humans. If genetic variants are identified in distantly related breeds of chickens or in other avian species, for example ducks, that confer resistance to bird flu, then GE technologies may be used to introduce these variants into production chickens. The mechanisms involved in the development of the chick embryo are very similar to those involved in normal human embryo development. Increased knowledge of the processes involved in chicken embryology will inform our understanding of normal human development and the changes involved in diseases, for example genetic diseases that affect the number of fingers on a hand or affect bone formation.. The laying hen synthesises large amounts of protein every day in the production of egg white. There is potential to harness this protein synthetic capacity by genetically engineering hens to produce large quantities of useful protein drugs in their eggs, for applications in human or veterinary medicine. The characteristics and functionality of such protein drugs will be further investigated, to evaluate this method of production and progress to testing specific molecules.
What species and approximate numbers of	The experiments will involve the domestic chicken, from embryos in eggs during incubation, through

animals do you expect to use over what period of time?	immature chicks to mature adult birds. We estimate that approximately 5500 birds will be used 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?	Any adverse effects will be due to the experimental manipulations of individual birds and, in a limited number of experiments, to the result of a specific genetically-engineered change that may affect the health of birds with this change. The experimental manipulations are most likely to reduce the proportion of chicks that hatch successfully as robust chicks, as embryos that are not robust fail to hatch, classified as mild level of severity. Some GE changes may affect the health of any bird carrying this genetic change, for example they may grow more slowly than non-GE birds or may have a reduced lifespan. For example, in investigations of genes involved in immunity GE mutation of these genes may reduce the health of the birds, indicating the functions of these genes. These birds will be monitored as appropriate for the particular debility and only bred if the scientific value of the experiments utilising these birds justifies this.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of the chicken as a model species itself addresses “replacement” to the extent that licensed experiments carried out in mice may be replaced by the use of GE chicken embryos from lines created under this licence, for studies in the early stages of development. Investigation of the development of mouse embryos also affect the mother as the mouse embryos develop within the mother’s uterus. Chick embryos develop in an egg and so investigations using chick embryos do not adversely affect the mothers. Investigation of aspects of the response to diseases, gene regulation in development (for example) can be carried out using in vitro systems and chicken embryos before the regulated stage of incubation (the regulated stage of incubation is the last few days before the chick embryos hatch, when they have developed to a stage where the embryos could possibly feel pain and discomfort), but comprehensive studies, for example of infection and host immune processes, are only possible in a whole animal and preclude the use of other models such as fish, flies and worms**.**
2. Reduction	Experimental designs will be developed with advice

Explain how you will assure the use of minimum numbers of animals	from a statistician who is a member of our AWERB. The number of birds required to maintain breeding lines of GE birds will be kept to a minimum (2-3 pens) required from our past experience of the numbers required to ensure continuity of a line. Birds of a GE line will be bred at greater numbers where specific project requirements need greater numbers of birds to be generated for limited periods, for example to test a novel vaccine in another project licence, in appropriate numbers of birds to estimate efficacy. When possible, GE birds will be bred to be homozygous for their specific genetic change, so that all their offspring are GE and as a result non-GE offspring, not required for experiments, are not produced. Discussions with colleagues developing and utilising GE mice will also ensure we benefit from advances in processes for ensuring numbers of animals used are kept to the minimum required.
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 chicken is both a farmed animal of major importance to human nutrition worldwide and a model organism for the study of vertebrate development. Increasing basic knowledge of the physiology and development of the chicken that can only be investigated in the intact embryo/bird, that involves understanding tissues and organs in the intact animal, is the focus of this PPL. Applications of GE technologies in biotechnology and genetics also must relate to the function of the intact animal. The studies are designed to be as minimally invasive as possible; the vast majority of birds will only be blood sampled to confirm transgenesis and then used for breeding as required. All new transgenic lines are closely monitored for any adverse phenotypes, working closely with our named veterinary surgeons.

Project 3	**Analysis of plant ion channel and receptors**	
Key Words (max. 5 words)	SNAREs / K^+ channels / cell growth / proliferation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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)	Potassium channels of animals, plants and fungi – so-called Kv channels – are very similar in structure, function, and their regulation. We know a great deal about the molecular mechanics of this regulation, but there are still many puzzles. Understanding Kv regulation is essential, because many of these channels are important in human and animal disease, in fungal pathogenesis, and in the environmental hardiness of crop plants. Channels also coordinate transport with membrane and cell expansion to regulate cell surface and volume. How such coordination is achieved remains a century-old problem in fundamental cell biology. From past work, it is now known that many channels bind and regulate proteins needed to drive membrane traffic. Again, what we don't yet understand is how this regulation is achieved. The knowledge from work with animals is rooted in the initial studies of the plant channels, and provides a strong argument to fundamental studies with the plant channels. The immediate challenges now are (1) to understand how channel binding with its	

	partners is coupled to channel regulation, and (2) to resolve the molecular dynamics of these binding events.
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)?	Previous discoveries of plant channel structure and of specific protein-protein interactions showed the importance of coordinating channel activity with cell expansion. These findings are now mirrored in recent work from animals. The past discoveries defined some of the key elements behind these processes, but they have also highlighted fundamental gaps in knowledge, notably of the dynamics of channel interactions with their partners. Filling this knowledge gap is needed to underpin research into many diseased states in animals, into the environmental hardiness in plants and, in both, into to the control of cell proliferation. The studies here aim to identify, characterise and quantify the interaction dynamics and the structures of the channels and their partners so as to understand their roles in the cell.
What species and approximate numbers of animals do you expect to use over what period of time?	Many aspects of these studies will be carried out using methods that do not require animals. However, controlled reconstitution of the channels together with the components relevant to cell expansion, and long-term functional analysis, requires that the proteins are tested in defined cell systems for which past work has established expression. Xenopus eggs have long been used for this purpose and provide an essential experimental benchmark. Use of Xenopus will be restricted to the harvest of eggs and will be limited a small number – not more than a total of 50 animals over five years – to provide essential insights that can be translated to analysis of protein function and its control in the plant and in non-living systems.
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 of the procedure are unexpected death under anaesthesia, post operative infection and wound breakdown. From previous studies in this laboratory, the unexpected death rate under anaesthesia is less than 0.1% and the rate of wound breakdown and post-operative infection is less than 0.2%. The severity of the operation is mild to moderate, and established measures are in place to minimize the risks to the animals. In all events, the toads will be terminated by a Schedule 1 method.
Application of the 3Rs	
1. Replacement	Use of *Xenopus* eggs is essential for the

State why you need to use animals and why you cannot use non-animal alternatives	electrophysiological element of the studies, especially for long-term recordings that cannot be achieved in other heterologous systems, including mammalian cell lines (eg. HEK cells) and insect cells (eg. Sf9 and Sf21 cells). Analysis of function requires that the proteins are expressed in vivo and in a defined heterologous cell system that is not a plant or fungus, since the latter will have these channels and/or related components, making defined analysis impossible. For these reasons, recording from eggs has been the benchmark for studies of this kind, and will be important for comparative purposes. For proper expression it is essential to use eggs at stage V or VI (late in development) harvested from *Xenopus* ovaries, as the the eggs become translationally quiescent following release and will no longer express proteins.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction will be aided by subdividing each egg harvest into pools and planning experiments to address parallel questions concurrently, as each harvest normally yields many more oocytes than can be used. Separate pools will be used to express and test a different protein construct or construct combination, thus reducing the total number of animals required. Measurements of ensemble and single-channel current will be recorded using established protocols (so-called two-electrode voltage clamp and patch clamp methods). Currents will be compared with known wild-type channel characteristics (temporal kinetics, voltage sensitivity, inactivation kinetics, ionic dependencies, etc.) and will be deemed satisfactory for any one construct using normal statistical standards.
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.	Use of *Xenopus* eggs, as noted above, is important for heterologous expression and analysis, and is the benchmark for studies of this kind. To minimize suffering, a single partial ovariectomy will be carried out under anesthetic with recovery. A second ovariectomy will be carried out only after terminal anesthesia and the animal killed by a Schedule 1 method, without regaining consciousness.

Project 4	**Phenotyping Genetically Altered Mice**	
Key Words (max. 5 words)	Phenotyping, Ageing, Genetics, High-Throughput	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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
	X	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 major challenge for genetics in the 21st century is determining the function of human genes. This project involves characterising mice which have had changes introduced into their genes (GA mice). One of the best tools we have to understand mammalian gene function is the laboratory mouse, which has been used extensively by the scientific community and which is fundamentally similar to humans at the genetic level. Whilst extensive work has been done to investigate gene function in specific systems in the mouse, there is still a need to understand the effects of a gene across the entire system. Using specifically designed tests, we will identify whether changes in certain genes cause abnormalities or disease and therefore investigate the normal gene function. The majority of the work on this licence will be part of the International Mouse Phenotyping Consortium (IMPC) whose goal is to provide a publicly-available database of gene function for the entire mouse genome. This service licence also provides a means to allow researchers to study the pleiotropic effects of a gene through a pipeline of standardised, validated and robust phenotyping tests in the hands of a skilled team of technicians.	
What are the potential benefits likely to derive from this project (how science could be	To date, the IMPC project has already delivered substantial benefits to the wider scientific community. These include phenotyping and expression data for	

advanced or humans or animals could benefit from the project)?	over 300 mouse lines which are fully accessible to the research community, scientific knowledge on gene function and disease including disease assignments with no prior functional information and internationally leading technical developments and support at Harwell which underpins other UK research including secondary phenotyping projects. This project license will seek to add to that wealth of information by continuing to investigate gene function in mouse lines through a broad-based phenotyping pipeline and disseminating that information to the scientific community. Furthermore, this project will also look at the effect of certain genes on ageing. In addition to this work, we will also be looking at genes of known function or thought to have a role implicated from current research and utilise the skills and technical expertise at Harwell to study the pleiotropic effect of the alteration of these genes in mice. These studies will be done in collaboration with researchers from across the UK providing detailed information on their genes of interest that could not be done elsewhere.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using the mouse exclusively for this work and we expect to use 132,600 animals over the course of five years. These lines will be chosen by the IMPC and the research community for the importance of the genes they carry.
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 first two protocols concern breeding GA mice. The first protocol is expected to not exceed a mild severity as mice bred under this protocol will have a known phenotype that is not expected to have an adverse effect. The second protocol concerns breeding mice with an unknown phenotype. A small proportion of the GA mice may have a moderate phenotype as a result of the GA. In these cases the mice will be much more closely monitored and advice will be sought from the welfare officer (NACWO) and NVS regarding the animal's welfare. The third and fourth protocols involve the testing of the mice through a pipeline of phenotyping tests. The majority of the phenotyping tests have a mild severity but one or two tests are of a moderate severity (e.g. intraperitoneal glucose tolerance test). All steps necessary will be taken to monitor the mice closely and prevent any adverse effects. At the end of the study the mice will be killed and tissues taken which will be required for the completion of the study.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary for us to work with living mice as the determination of an in vivo phenotype requires a multi-system, comprehensive assessment that can only be done within an intact body system. The pleiotropic effects of genetic alterations that are often seen would be missed if an isolated organ were studied in vitro. Moreover our experiments require manipulation of the genome to understand gene function. The mouse shares similar physiology and anatomy to humans and in addition the mouse genome is known and genetic manipulation is possible in mice, mouse is therefore the best model system for these studies
2. Reduction Explain how you will assure the use of minimum numbers of animals	Genetically altered (GA) mouse lines will only be maintained whilst there is a justified use for their continued breeding otherwise lines will be cryopreserved and removed from the shelf. Prior to establishing a new colony of GA mice under this licence, any available breeding data will be sought and well established breeding calculations will be used to predict output. In the case of lines of unknown viability, advice will be sought from experienced animal technical staff. For larger, complex multisystem projects, experimental numbers have been determined in collaboration with expert statisticians. A mixed model approach has been used to determine the minimum number of mice that could be used for each test within a high-throughput phenotyping pipeline. The numbers of mice used for each test is continuously reviewed at biannual meetings and the data is constantly interrogated to determine whether the n numbers are appropriate. For smaller projects, power calculations are typically done based on wild type data collected previously for each test to determine the numbers that should be used. We constantly interrogate and analyse the data to determine the variability and where possible we work to reduce the variability as this will ultimately result in decreased numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The mouse is the lowest mammalian species in which the full range of genetic manipulations necessary for the investigation of gene function can be achieved. It is critical to perform these studies in mammals as are interrogating mammalian gene function and other

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	animal and non-animal species cannot supplant studies specifically in mammals.Welfare assessment will be continually undertaken throughout the lifetime of the mice and where possible non-invasive phenotyping will be performed.Phenotyping tests that are employed will be reviewed continually to ensure that they are the most refined to provide the most advanced scientific outcome.

Project 5	**Spatial and temporal regulation of DNA replication**	
Key Words (max. 5 words)	DNA replication, cell proliferation, chromosome, nucleus	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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)	One key feature of life is the capability of reproducing, essential for single cell simple microbes through to complex multicellular organisms. The basic form of reproduction is the capability of a cell to copy itself, giving rise to identical daughters. This process is based on the copying and transmission of the genetic information contained in the DNA. This process is called DNA replication and it is highly regulated, as mistakes will inevitably lead to either death or the wrong information being passed on. We are interested in understanding some aspects of the control of DNA replication, such as its temporal and spatial regulation. Generally, the more complex is an organism the bigger is the size of its DNA. However, the time to entirely copy the DNA should be the same for all the cells, given that the number of sites from which DNA replication starts is proportional to the size of the genome. Nevertheless, this is not the case, and DNA replication takes place according to a specific program, the DNA replication-timing program, that controls which part of the genome replicates where and when. Why that is still unclear. However, this	

	regulation is dynamic during embryonic development and altered in pathological situations, suggesting a probable fundamental role of the DNA replication-timing program.
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 understanding of the molecular control of basic cellular processes such as DNA replication is fundamental to the comprehension and treatment of pathological situations where these processes are altered. DNA replication is the main, inevitable and constant source of cellular damage from within the cell and its deregulation can be both the Achilles' heel as well as the base of how cancer starts and develops. Having identified the molecular components that control the DNA replication- timing program, we will be able to induce its deregulation in a controlled manner and dissect its role during cancer development.
What species and approximate numbers of animals do you expect to use over what period of time?	Our studies use the mouse as a model system because, like humans, it is a multicellular organism, with a physiology, embryonic development and organization of the genetic information comparable to ours. Unlike with humans, however, the genetic information in the mouse can be manipulated. This allows the perturbation of physiological and cellular processes and thus the understanding of their functioning. The mouse is therefore the most advanced genetic mammalian model where gene function and contribution to disease and development can be understood. We project that 15000 mice will be generated by breeding over a period of 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 majority of the mice will be used for breeding to assemble the desired combination of gene variants to explore the research objectives. We will then mate the animals with the appropriate genotype to obtain cells from embryos at very early stages of development (from E3.5 to E12.5). Our protocols do not imply physical pain or discomfort, since the experimental mice are sacrificed by humane methods and only afterwards the early developing embryos are isolated in order to obtain cells. We rely continuously on the animals as inevitable source of different types of embryonic cells. Unfortunately, the majority of primary cells have only a finite life-span and we have therefore to isolate them fresh from the source every

	time.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research aimed at investigating the molecular mechanisms of proliferation in mammalian cells can avail either of the use of cell lines or primary cells. Cell lines have the advantage of been maintained indefinitely in culture, without the need for a renewable source of cells. However, these cells are able to proliferate because they have undergone various degrees of genetic changes that make them cancer cells, Studies conducted in this system have therefore a limited value in terms of understanding the functioning of the basic and normal molecular machinery. Primary cells, on the other hand, reflect the biology of cells of the mammalian organism, but they have a very limited life-span in culture and therefore require a constantly renewable source. Regulation of DNA replication in primary cells is fundamentally different then in cell lines, as the process of immortalization severely affects DNA replication. We therefore inevitably have to rely on animals as a continuous source of genetically engineered primary cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to reduce animal usage we will apply optimized experimental designs, statistical analyses and breeding strategies. Optimized experimental design will be employed to maximize the information from each animal and thus limit the subsequent use of additional animals. We will maximize the number of assays run in parallel from each round of cell isolation and use power calculations to reduce to a minimum the number of animals used in each experiment.
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.	Since we study a process that is fundamental to cell survival, our work is based on the generation of alleles that can be conditionally and rapidly inactivated in cells. This approach has a double advantage. Firstly, the allele functions normally in the animal and we can induce its inactivation after cells have been isolated. Thus, there is no need for breeding animals with ill-health. Secondly, the controlled and acute inactivation of a gene enables the study of its function without the confounding effect of adaptation. We are also interested in the regulation of DNA replication in space and time during embryonic development, a time in the life of the fast-

	growing organism when this process is especially challenged. Thanks to the technical developments of gene manipulation and the extensive developmental studies, the mouse is to date the best system for achieving both our aims. Our procedures do not imply suffering and there is no protocol categorised as severe.

Project 6	**Functional analysis of vertebrate enhancers**	
Key Words (max. 5 words)	Gene regulation, enhancers, conserved sequences, development	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		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)	The aim of this project is to understand how genomic sequences can direct gene expression during early development of the vertebrate embryo. We have already used computational approaches to identify a specific set of non-protein coding sequences that are found in all vertebrates. We have termed these Conserved Noncoding Elements (CNEs) and we know that a majority of these can activate gene expression in a controlled way during development. Currently however, we have no way of telling exactly when or where in the embryo any chosen CNE will work and to do this, we have to crack the 'regulatory code' within the CNEs. For example, we have recently identified a piece of DNA grammar activates genes in the developing hindbrain, but to prove this we needed to test a number of candidate CNEs in living embryos. We have developed and refined a way in which we can monitor when and where genes are turned on using a fluorescent protein that lights up under the microscope. Generally, we perform this	

	on zebrafish embryos up to 72 hours post fertilisation. However, in some cases, when the signal is unclear, it is necessary to make a permanent, “transgenic” line that incorporates the fluorescent protein into the genome of every cell in the embryo. Our aim is always to minimise the number of transgenic lines, and fish, that we need to establish. Furthermore, the purpose of making these lines is to observe gene activity in ‘normal’ embryos. We therefore screen fish for a few healthy individuals before the free-feeding stage and cull all others. Zebrafish are ideally suited to this project as they lay eggs in the water, easy to keep and breed, and embryos are essentially transparent and can be screened and monitored before they are free-feeding. As far as we are aware, zebrafish tolerate our fluorescent gene without any deleterious effects. In parallel, my laboratory carries out complex computational analyses to help us understand regulatory DNA. This ensures that we generate clear hypotheses that predict the function of some CNEs which in turn reduces the number of *in vivo* experiments we need to perform. The output of this work will provide a better understanding of how and where CNEs drive gene expression in the developing vertebrate embryo, and in conjunction with other projects in the lab, will permit prediction of regulatory element function, thereby reducing the amount of experimentation necessary.
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 output of this work will provide a better understanding of how and where CNEs up-regulate gene expression in the developing vertebrate embryo, and in conjunction with other projects in the lab, will permit prediction of regulatory element function, thereby reducing the amount of experimentation necessary. We are also constantly striving to improve the transient assay we use so that making permanent transgenic lines is an largely unnecessary.

What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish Up to 10,000 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?	Mild severity limits. The transgenic lines we do generate appear to thrive as well as wild type animals. We only perform gamete recovery rarely and this is also a gentle and mild procedure. Animals, like their wild type counterparts, are culled at about 18 months.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have no way of predicting where a regulatory element exerts control over gene expression unless we can observe it in an animal
2. Reduction Explain how you will assure the use of minimum numbers of animals.	Wherever possible we use transient analysis on early (pre-free feeding) embryos that are subsequently culled before they reach 5 days old.
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.	Zebrafish are oviparous, avoiding undue stress to The adults. They produce large numbers of embryos easily and naturally. In conjunction with the aquatics unit we strive to ensure any handling of animals is both minimised and done under the optimal conditions for the animals concerned.

Project 7	**Advancing methods for rederiving and archiving genetically altered mice**	
Key Words (max. 5 words)	Mouse, freezing, sperm, embryos, technology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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
	X	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 publication of the human and mouse DNA (a molecule that makes up genes) sequences, coupled with the availability of an array of sophisticated tools make it possible to alter any specific gene of interest. This in turn has led to the potential to understand the function of every gene in the body. Understandably, investigators want to tap into this potential and they like to use the mouse as a model for understanding gene function in man. Investigators also like to be able to exchange the mouse lines they work on with other colleagues. Freezing embryos and sperm ensures unique mouse strains can be made available to future generations of scientists This project has been designed to refine the technologies used for the efficient freezing (embryos/sperm) and recovery of genetically altered strains of mice. These strains will be transferred to projects that have been set up to investigate specific	

	aspects of mammalian biology. By combining technology development with a central service for mouse archiving and distribution we will be able to invest in the most up to date equipment/techniques and get unique mouse models of human disease to investigators quickly. By developing new techniques and training our staff in these techniques we can maintain our proficiency in cryopreservation and recovery methods. We will also make sure these new advances are widely published so that others may improve their procedures. By doing so we will ensure that we (and other resource centres) provide an efficient service to the scientific community. In doing so we will help to reduce animal numbers and improve animal welfare.
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 benefit science by advancing the methods used for the freezing, recovery and distribution of genetically altered mouse strains used to better understand how genes control all aspects of mammalian biology. In particular, this project will help scientist around the world by publishing the technology advances we make on websites and in scientific articles. This will promote use of better techniques and reduction in animal usage.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will last for 5 years and will only use mice. It is expected that up to 9,500 animals will be used to support the technology development work proposed in 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 happen to the animals at the end?	The techniques used in this project have been designed to minimise suffering and animal numbers. The techniques are all well established and the majority involve no more than mild discomfort e.g. hormone injection or ear clipping. What is more the incidence of adverse effects is known to be low. Each animal line is examined comprehensively throughout its life-span for indications of ill health. If a mouse is exhibiting detrimental signs (such as weight loss) then the mouse will be killed. No animal will be kept in a prolonged state of suffering.

	Embryos will be transferred using an operation that will be performed under general anaesthetic and pain relief will be given. Ear clips will be taken in order to confirm which mice carry the gene of interest. This procedure is only associated with momentary discomfort. Similarly, embryo production will be facilitated by injecting hormones which is a technique that also only induces momentary discomfort. When mice are mated, care will be taken to ensure that the mice are mature enough to mate. Any over vigorous males will be removed from the mating The overall severity limit of this project is expected to be moderate Mice used in this project will either be transferred to another project for further study or humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Model organisms are the key to working out the function of genes and proteins. We are now able to manipulate genes using genetic engineering and investigate the consequences for the whole animal. Animal models, such as the mouse, present scientists with a unique opportunity to uncover the function of genes and the genetics of disease. Although we will be able to cross reference existing databases and published methods non-animal models cannot be used for this project because we are trying to improve the efficiency of methods used to specifically freeze down or recover live mice. These are essential techniques that in themselves reduce animal usage and suffering. We recognise that databases, cell culture systems and non-animal models are often used to study biological systems but many scientists need to know how particular genes affect complex organ systems like the heart, brain and lung. At the present time there are no cell culture systems available that can provide these results.
2. Reduction	Before starting any study in this project, data will be

Explain how you will assure the use of minimum numbers of animals	collected from any previous relevant studies and statistical analysis used to make accurate predictions of how many animals we will need to produce a decisive scientific result. In order to keep the number of animals to a minimum, only mice required for such studies will be bred. The efficiencies of all techniques used in this project will be subjected to regular audits to ensure consistently good results whilst striving for improvements. The idea behind establishing a technology development project is to establish a pool of skilled people with expertise in the latest techniques so that they remain proficient and can pass on their skills. Thus keeping animal usage to a minimum at all times. We actively promote the cryopreservation of new mouse strains by all members of the scientific community. This eliminates the need to continually breed genetically altered mice when they are not needed in active research programs. Freezing embryos and sperm also preserves these unique strains of mice for future research.
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 mouse is the most appropriate animal model for this project because we support scientists who's intended aim is to work out the function of all mammalian genes and proteins. Animal models are important because we are able to manipulate their genes using genetic engineering and investigate the consequences for the organism. The mouse occupies a unique position in determining gene function and the genetics of disease for a number of reasons. Firstly, as a mammal it demonstrates a remarkably similar development, physiology and biochemistry to the human. Secondly, mouse geneticists have developed a very extensive genetic toolkit that enables very specific alteration of genes in the mouse. Thirdly, we now know the complete sequence of all the DNA the mouse carries. We will minimise the welfare costs to the animals by using the minimum number of animals at all times. We will constantly reviewing the techniques we use

	so that we introduce new refinements at the earliest opportunity.

Project 8	**Molecular genomic and phenotypic analysis of environmental adaptations in Cyprinid fish**	
Key Words (max. 5 words)	Molecular genomic, phenotypic analysis, environmental adaptations, Cyprinid fish	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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)	Exactly how do animals protect themselves from the potentially damaging effects of environmental fluctuations and the seasonal extremes of temperature and oxygen availability? We know that many species have the ability to 'acclimatise' their bodies to changed environmental conditions so that they prosper better than had no change taken place. This is part of the normal repertoire of responses by animal species that inhabit fluctuating environments and is most evident in species from extreme habitats. The underpinning mechanisms of response are known in some species and in relation to some specific environmental factors. But very little is known about how vertebrate animals respond to seasonal cold in the winter, particularly the cold blooded animals such as fish whose body temperature is set by that of its immediate environment. The same is true regarding changes in the provision of oxygen in aquatic environments, and lethal hypoxic zones are increasingly evident in both	

	freshwater and marine environments. Some aquatic species experience daily period when oxygen is difficult to obtain, a good example being the common carp. This project is designed to provide new information on the molecular basis of environmental tolerance. First, we will take advantage of new genomic technology to uncover the kinds of proteins that are prioritised for synthesis as the effects of cooling occur, and we will relate this to the kinds of gene transcripts being generated. This analysis will give a strong indication of what the processes are that contribute to environmental resilience. A more precise understanding of this protective process can provide insights of relevance to all vertebrate animals since they share many of the same regulatory features in their cells. This fundamental understanding might lead to novel ways of handling the treatment of conditions in animals and humans. Second, we will sustain an interbreeding population of genetically altered zebrafish, harbouring a genetic knockout of what we believe to be a key respiratory gene that underlies resilience when oxygen becomes a limiting resource for life. Further technical developments of this experimental resource will allow us to investigate the potential role of this extraordinary gene in tolerating low environmental levels of oxygen.
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 work will lead to the detection and definition of currently unexplored molecular/cellular responses to environmental challenge, which are known to induce protected states, resistant to injury and death. Just as the discovery of heat shock proteins 30 years ago has revolutionised our understanding of disease as well as environmental tolerance, the discovery of chill tolerance mechanisms is likely to offer insights into other properties of animals and humans, including the diagnosis and therapies directed at human and veterinary diseases.
What species and approximate numbers of animals do you expect to use	We will focus mainly on the common carp, given its ability to display tolerance responses. We shall use approx. 300 individuals over a 3-year period.

over what period of time?	For the development of a zebrafish model for assessing the importance of a key gene, myoglobin, we shall develop an interbreeding population of genetic knockouts. This comprises approx. 500 adults and 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?	For the cooling experiment we propose to expose the carp to programmed cooling of their aquarium water. This will be done slowly and with several periods when the temperature is maintained constant to help them readjust, as happens normally with the seasons of the year. Whilst rapid cooling can generate aberrant behaviour and a loss of coordination and other signs of distress, we know that the slow, intermittent cooling as in our protocol will not elicit any such signs. This procedure is mild in its severity since the animals do not display and behavioural signs of effect. At specific times after cooling the fish will be killed by the Schedule I method and tissues sampled for analysis. The zebrafish population will not be exposed to any treatments since we are just maintaining a breeding population.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are exploring tissue responses that are specifically generated within the context of the whole integrated animal, as controlled by its complex neuroendocrine regulatory systems. This cannot be done with any confidence in cell or tissue cultures, particularly as some responses take several weeks to become evident.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use careful statistical designs that minimise use of animals for given set of outcomes. This is based on our extensive prior experience of this experimental system, and the well defined level of variation in responses between individual fish.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Of all animals with backbones, the common carp has the amongst greatest ability to develop a tolerance to naturally occurring thermal and low oxygen environments. Thus discovery of the underpinning process of adaptations are most clearly defined in

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	resilient species. Some species live is environments that are relatively constant and they do not display such responses to the same extent, a good example being the rainbow trout. Welfare costs are minimised by (i) use of careful animals rearing and husbandry techniques, (ii) making sure that all of the animals are in all respects healthy and preconditioned for months to the starting temperature, (iii) employing well established exposures protocols, known to lie within the evolved limits of each species for adaptation and survival; (iv) optimising experimental design to minimise the number of protected animals used to give an acceptably define experimental outcome.

Project 9	**The European Xenopus Resource Centre**	
Key Words (max. 5 words)	European, Xenopus, Resource, Centre	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Xenopus are one of the most powerful animal models that are used to understand human and animal diseases. This needs genetically altered and inbred animals which are collected, made, quality assured and sent to laboratories all around the world.	
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)?	Research using Xenopus benefits human and animal health in two ways; first by discovering the basic mechanisms by which cells work and interact with one another and second by being used as models of human genetic diseases. The results from experiments using Xenopus underpin much of our understanding of important diseases such as cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	All of the work will use African frogs Xenopus species). Some 3850 adults and 32000 tadpoles will be used over a 5-year period.	
In the context of what you propose to do to the animals, what are the expected	Most of the adult animals will be used to lay eggs as a result of a hormonal stimulation administered by injection. This is a mild severity procedure and the	

adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	frogs can be re-used 15 times before they are finally killed humanely and their oocytes used for experiments. Up to 250 adults will act as “surrogate mothers” for experimentally manipulated oocytes. These have oocytes implanted into their abdomens through a large needle whilst under anaesthetic, which is a moderate severity procedure. These animals are killed humanely after the experiment. The tadpoles carry specific, targeted mutations in their genome and since some of the mutations are likely to be harmful we must assume that this is a moderately severe procedure. Tadpoles that develop abnormally will be killed humanely and analysed. The animals that develop normally will be allowed to grow to adulthood and used for breeding.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Xenopus researchers study cell interactions and how cells behave in the whole organism and this requires embryos. These in turn need to be produced by adult animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimise the use of adult Xenopus by carefully planned sharing of embryos between projects. We will attempt to reduce the numbers of male Xenopus used by freezing sperm, allowing 8 experiments to be performed using each male rather than 2-3 as is currently the norm.
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 minimise the harm to animals we are using a new technique to re-introduce oocytes into female Xenopus; surgery has been replaced with reintroduction using a syringe and needle. We are also developing an approach that will allow us to perform the experiments that normally need oocytes to be re-introduced to females entirely without that process.

Project 10	**Early growth and life-long health**	
Key Words (max. 5 words)	Development, growth, genetics, epigenetics, metabolism	
Expected duration of the project (yrs)	5 Yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to understand how growth is regulated during the early phases of life, particularly the critical periods of gestation and lactation. In man and other mammals early growth relies on interactions between mother and offspring through the placenta and mammary gland. Although growth is a fundamental part of our biology the genes and mechanisms that regulate growth are still poorly understood, particularly at the level of tissues, organs and the whole body. Animals typically grow to a size that is optimal for survival and involves both genetic and environmental factors. Importantly for mammals, this includes interactions of genes in both the mother and offspring. At the same time the environment, including maternal nutrition, influences offspring growth. Uncontrolled growth is linked with rare syndromes in which body size and proportions are incorrect, and also with cancer. Poor growth during the early stages of life have also been linked with life-long health status, greatly increasing the risk of	

	common metabolic disorders including obesity, diabetes, heart disease and also some aspects of mental health. This has been termed 'developmental programming' of health. So far, no genes have yet been proven to link early growth regulation and long-term health, but we have identified two strong candidate genes. Our main objectives are: To prove that one or both candidate genes have key roles in developmental programming of health. Use genetic techniques to identify further genes that act together with the candidates in developmental programming. Identify how and where the developmental programming factors act, including in the placenta and mammary gland, the key energy storage tissues (adipose, liver muscle and pancreas), and tissues known to exert central control on energy metabolism (such as the hypothalamus of the brain and associated pituitary gland). Determine how programming factors act to influence growth, lean:adipose body proportions, behaviour and might thereby contribute to disorders of growth (including cancer), metabolism and mental health.
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)?	By understanding the genes and processes underlying developmental programming we expect to contribute to understanding of major health issues. Obesity alone is a major global health threat with around 60% of people worldwide classed as obese or overweight. In developed and developing nations prevalence is increasing, notably in children, and is projected to continue. Direct health care costs for overweight and obesity are estimated at >25% of total EU healthcare spending (the wider costs to patients and carers are much greater). Obesity is a complex condition and developmental programming may make a significant contribution to this burgeoning heath problem, as well as other prevalent disorders, including diabetes, heart disease, cancer and anxiety-related mental health problems.

	Understanding of the causes will help identify those at risk and pave the way for new interventions (potentially including dietary advice or supplements during pregnancy) and treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6,000 mice 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?	Many of the mice carry defined genetic mutations that affect growth, lean:adipose body proportions, energy metabolism and behaviour. Most will experience only mild adverse effects as a consequence of their genetic alterations, e.g. they may be leaner or fatter than normal. Some may be at increased risk of developing cancers but will be killed before or as soon as tumours become apparent. Milk will be collected from some lactating females under anaesthesia. Some mice will undergo tests for their ability to store and use glucose, or to respond to insulin, which involves injections and collection of blood samples, usually after an overnight fasting period. Mice may need to be placed in a restraining device for a few minutes while taking blood samples, blood pressure readings or for body scans (e.g. NMR or micro-CT scans). Animals will be anaesthetised for scans where they must be still. Some mice will be subject to simple behavioural tests that may cause stress, e.g. due to being housed alone (sometimes for days or a few weeks when undergoing a series of tests), placed in an unfamiliar environment. Mice may also need to be housed alone for several days in high-tech cages that measure their activity, food and water intake and energy expenditure. Most of the outlined procedures cause at most, transient pain or distress, which is considered mild harm. The exception is for those animals housed singly for longer periods (weeks) which may be moderately harmful. When animals experience multiple tests the total will not exceed moderate harm. Animals that become ill during any protocol will be humanely killed – this includes those that develop overt symptoms of illness, sustain significant injuries that cannot be

	remedied successfully using minor interventions, or suffer 10% loss of weight within a week. At the end of the experiment mice will be humanely killed and usually a range of tissues taken for analyses that are part of our work.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to understand growth during early life and subsequent effects on metabolism, longevity and mental health. We do use cell culture where possible, but ultimately there is no substitute for the whole animal as we study gene function at the level of tissues, organs and the whole organism and developmental processes rely on interactions between mother and offspring that are unique to mammals (mediated by the placenta and mammary gland).
2. Reduction Explain how you will assure the use of minimum numbers of animals	We keep up with developments in the field to avoid duplication of experiments and use knowledge derived from complementary technologies (including our own in vitro studies). Apply statistical analysis routinely to estimate minimum numbers of animals required for valid comparisons. Power calculations/curves are used to project numbers, and are required by all major funding bodies. Plan genetic crosses efficiently to generate animals of the desired genotypes, including appropriate controls (typically littermates matched for age, sex, genetic background and environment, including anu influences of the mother). Collect multiple samples from the same animals, where possible in longitudinal studies and/or after animals have been humanely killed. Pool data from different time-points (when appropriate) for statistical analysis. Use statistics, modelling and network analyses for optimal use of the data we obtain.

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.	Our work is specific to mammals, including understanding of the critical early-life growth periods involving interactions between mother and offspring and how these impact on physiology and behaviour of the whole animal. Therefore, the work cannot be carried out in non-mammalian animals or purely *in vitro*. Among mammals, mice provide the optimal system to combine prior knowledge of genetics growth and physiology, with the ability to manipulate gene and determine their function. We always seek to minimise exposure to stress, harm or pain, e.g: minimising periods of isolation and fasting, without compromising experimental validity. Appropriate administration of anaesthesia and pain relief. Use careful monitoring to detect harmful defects (such as cancers) and alleviate suffering at the earliest opportunity.

<table>
<tr><td>Project 11</td><td colspan="2">Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Cardiovascular and Metabolic Disorders Diseases and Their Treatment</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">NADPH oxidase; oxidative stress; cardiovascular diseases; insulin resistance; diabetes</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Cardiovascular disease is a major cause of death and illness in people after middle-age and in people with obesity and diabetes. Novel therapeutic targets to treat these diseases are urgently needed to save lives and to improve our life expectance at older age.

The cells in our body generate reactive oxygen species (ROS) as products of their metabolism. Under normal physiological conditions, the amount of ROS produced by cells is tightly controlled and eliminated quickly from our body. However, when the cells face challenge or in a diseased environment, they generate too much ROS (oxidative stress) that cause damage and cell death. Discovery of the major sources of ROS generation under diseased conditions is crucial for the development of new therapies. Recently, an ROS-generating enzyme called NADPH oxidase has been found to be involved in the oxidative damage of blood vessels and the heart, and in the development of metabolic diseases. Therefore, the</td></tr>
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	overall objective of this project is to discover the mechanism of NADPH oxidase activation and to test NADPH oxidase inhibitors for the purpose to treat oxidative stress-related cardiovascular and metabolic 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)?	This project is to discover how the ROS are generated by the NADPH oxidase in cardiovascular and metabolic diseases, which cause damage to our health. The ultimate goal is to treat oxidative stress-related human cardiovascular and metabolic diseases. The information from this project will contribute and advance our understanding of the role of NADPH oxidase in the development of cardiovascular diseases. The NADPH oxidase inhibitors have great potential for the development of novel therapies for human diseases and will benefit our life and society.
What species and approximate numbers of animals do you expect to use over what period of time?	We are proposing to use rodents (mainly mice) for this research project. Approximately 2500 animal are budgeted over the 5 year duration of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Although there are adverse effects such high blood pressure, obesity and insulin resistance associate in these animal model of human diseases, inhibition of ROS production is expected to provide protection to animals from oxidative damage and reduce symptoms of cardiovascular and metabolic diseases. The expected level of severity is mild or moderate. At the end of experiments, animals will be culled by schedule one procedure and organs will be collected for biochemical and path-histological examinations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternative ways (such as cell culture, computer modelling and organ functional assessment) have been carefully considered and used anywhere it is possible. However, ROS metabolism in out body is a complex process that requires integrated actions of many organs/tissues and hormohes in a living animal or human. It is not possible to study or to inhibit ROS generation in the cardiovascular system without using living animals. This is currently the

	only way to achieve this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will be designed taking into account statistical concepts, such as power and precision, where appropriate, to minimise the number of animals required while yielding statistically sound results. More generally, when necessary, or appropriate, a professional statistician will be consulted to ensure an experimental design is optimal and minimises the number of animals required, yet ensures an adequate level of precision and power, and the appropriate statistical analysis is performed. Experimental design factors such as randomisation and level of replication will also be considered. We encourage the share of animal organs between investigators. Tissues obtained from the animals will, wherever possible, be used in several sets of experiments such as for cardiac steIn cell isolation (heart), for vessel function (aorta), for bone marrow cell isolation (legs) and to reduce the number of animals to be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mainly mice for this project. Mice deficient of Nox enzyme are available and have been used in our previous projects. These mice produce less ROS in diseased conditions, which provide protection against oxidative damage and reduce symptoms. Where the immune status of these mice makes them susceptible to bacterial infection, animals will be maintained in a clean and appropriate environment and inspected daily. If there is any sign of infection, animal will be culled immediately by schedule procedure. We encourage the use of cuff-handling technique whenever possible to minimise animal stress. For the study that requires the delivery of drugs to animals, we will choose the way to achieve the best results with the minimum pain and suffering to animals. Appropriate anaesthetics will be used for any surgical procedure, and analgesia will be given post operation, and animals will be closely inspected after operation. If there is any sign of an animal under suffering, NVS will be consulted and animal will be culled by schedule procedure one procedure.

Project 12	**Effects of contaminants on fish physiology**	
Key Words (max. 5 words)	Fish, metals, contaminants, toxicology, physiology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
	x	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 the project are to better understand the uptake, metabolism, excretion and physiological consequences of contaminant, which includes, metals, metalloids and emerging synthetic contaminants (pharmaceuticals and pesticides) exposure to fish.	
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)?	There are three main benefits from this project. 1. conducting whole animal studies to validate our cell culture models will potentially result in our cell culture models being adopted as replacements for whole animals in bioaccumulation and toxicology studies reducing the numbers of fish used in future tests. In addition to reducing numbers of fish used it has the potential to be a cheaper alternative to whole animal testing testing and would thus benefit industries that have to re-evaluate the risk posed by chemicals they produce under REACH (Registration, Evaluation, Authorisation & restriction of Chemicals) legislation and conduct environmental risk assessment for new products. However, for wider	

	acceptance OECD approval would be required and a round robin testing procedure would take between 5 - 10 years. 2. investigating the adverse effects of contaminants on fish and thus have relevance for environmental regulations necessary to protect ecosystem function. It is highly unlikely that a single experiment would have the capacity to change aquatic environmental regulations and thus the results from this project are more likely to contribute to weight-of-evidence approach to regulations. 3. using fish as a model to investigate metal homeostasis benefits the aquaculture industry and society. Fish from aquaculture accounts for approximately 50% of the protein consumed worldwide and is likely to rise as the world's population increases along with the demand for protein. Improving production methods in aquaculture will help meet this demand. An area of current research interest is improving the availabilty of micronutrients (e.g. metals) in the commercial diet of fish. An improvement in the bioavailability means less metal is added to the diets, this reduces the cost to the aquaculture industry and reduces the release of metals into the environment from uneaten food or faeces.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately: Rainbow trout (*Oncorhychus mykiss*) - 1250 Brown trout (*Salmo trutta*) - 750 Atlantic salmon (*Salmo salar*) - 750 Zebrafish (*Danio rerio*) - 4000 Stickleback (*Gasterosteus aclueatus*) - 750 Medaka (*Oryzias latipes*) - 1000 Fathead minnow (*Pimephales promelas*) – 500 over the 5 year duration of the project
In the context of what you propose to do to the animals, what are the expected adverse	The protocols described cover: 1. Exposure to contaminants and metals and measurement of whole animal cation or contaminant

effects and the likely/expected level of severity? What will happen to the animals at the end?	uptake and/or elimination during. The fish may be exposed for up to 12 months, this is chronic exposure and is thus classed as moderate severity level. Exposure concentrations are sublethal and are based on previous published data or reported no-effect concentrations in chronic toxicity studies. At the end of the procedure animals will be humanely killed. 2. Cannulation of a major blood vesse(dorsal aorta)l or intestine to study metal or contaminant uptake and metabolism across the gill and gut. The procedure involves surgery to insert dorsal aorta cannula or insertion of cannula into the intestine to allow perfusion and is classified as moderate. For the dorsal aorta cannulation surgery excess bleeding may occur around the insertion of the cannula if this occurs then alginate fibres would be applied to encourage clot formation. The fish may also dislodge the cannula so samples may not be taken. If excess bleeding cannot be stopped or cannula is dislodged then fish will be humanely killed. There is risk of infection during and after the intestinal surgery. Application of antibiotics in the wound and topical in the form of a paste will reduce the chances of infection. If it does occur, as evident by haemorrhaging in the dorsal fin, fish will be humanely killed. 3. Measurement of reproductive output following exposure to contaminants. This procedure is mild severity. However, fin clips for genotyping may be required and this has the potential to cause discomfort thus this procedure would be perfomed under anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whole organism uptake and and metabolism of metals and contaminant is a complex physiological process.Cell culture or tissue slices, do not have the full endocrine or nervous inputs that may control theses processes, and thus in certain instances whole organisms studies maybe necessary to fully understand physiology. .

	We have made substantial efforts to replace animal experimentation with alternative methods. This has for example resulted in development of a laboratory based gill cell culture system, which we shown to mimic the response of the whole animal to metals and uptake of pharmaceuticals from the water. To validate this in vitro method it is necessary to perform some in vivo studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Long-term exposure studies to assess uptake and elimination of compounds is traditionally conducted on single compounds. However, recent studies suggest exposure to mixtures of compounds may provide similar results and this strategy will be employed where appropriate. For the flux experiments Power analysis (http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html) will be used to determine the number of animals to be used in the experiment. The *in vitro* models developed, may also reduce the number of animals used in experiments.
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 fish species to be used will depend on the parameters to be measured, for example, zebrafish will be used in a number of metal transport study because there are a number of genetically altered zebrafish strains for these these transporters; the gill cell culture has been developed using rainbow trout thus this species will be required to compare in vivo and in vitro results; rainbow trout, brown trout or Atlantic salmon, are commercially important fish species and understanding metal uptake processes in these species may assist aquaculture feed companies in designing diets with more readily available metals; zebrafish, medaka and fathead minnow are species with international standard reproduction tests protocols and thus results from the current project can be directly compared to those from other groups using the same species. Refinement during surgery includes using appropriate anaesthesia for the species of fish throughout the procedure, a water flow through table for surgery to maintain the fish moist and an injection of non-

	steroidal anti-inflammatory drug to reduce pain for up to 48hrs. To prevent infection antibiotics are administered to the wound prior to suturing.

Project 13	**INVESTIGATING THE (PATHO)PHYSIOLOGICAL IMPORTANCE OF S-ACYLATION**	
Key Words (max. 5 words)	S-acylation, palmitoylation, zDHHC enzymes	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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
	X	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 research are to understand how physiological processes are regulated by the attachment of fats onto cellular proteins (a process termed “S-acylation”). As defects in S-acylation are linked with many important diseases, such as neurological disorders, cancer and diabetes, it is important to understand the underlying basis for this so that new medications and therapeutics can be developed.	
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 work will lead to an important advance in our understanding of protein S-acylation. This is a widespread but poorly understood process, and our research will lead to a greater understanding of its role in physiological pathways. Furthermore, as defects in S-acylation are linked with diseases such as cancer, diabetes, Huntington’s diseases and schizophrenia, this research may also lead to a better understanding of the underlying pathology of these diseases as well as the	

	development of new and more effective medicines.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use approximately 3,000 mice over a 5-year 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?	We expect any adverse effects on the mice to be minimal, although we cannot state this with certainty as we will be using novel genetically-modified mouse lines. However, we know that inactivation mutations in zDHHC9 whilst causing intellectual disability are not otherwise lethal in humans. Caveolin knockout mice are viable and fertile and hence we do not expect any major adverse effects caused by mutating the S-acylation sites in this protein. After behavioural/physiological measurements are taken mice will be humanely culled and if appropriate, tissue may be taken for in vitro studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal usage is essential to allow us to examine the physiological effects of disrupting protein S-acylation. It is not possible to study higher integrated processes such as learning and memory using cell-based assays, and simple model organisms lack the required complexity and physiological pathways to allow a detailed understanding of the many roles of S-acylation in humans. Mouse lines are the most frequently used animal model to study the importance of specific genes/proteins for physiology and to provide appropriate disease models and their use in the current project is essential. Where possible, we will complement these studies by using cell lines however the use of animals is essential to achieve the aims of our investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure the use of minimal numbers of animals by careful experimental design. Thus, we will use the same animals in multiple behavioural tests and physiological measurements where this can be achieved without causing undue stress to the animals and without adversely affecting the outcome of experiments. Similarly, we will take any physiological

	measurements and tissue samples from the same mice, where this is appropriate.
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 will be used as they are the most common mammalian species used for analysis of behaviour and physiology following genetic manipulation. Thus, they are the most appropriate model to investigate the importance of S-acylation for these processes and to provide a model for human disease processes. A C57BL6 genetic background will be used as standard for analysis of genetically modified mice. As many behavioural tests can be used that measure Animals will be housed, bred and maintained following best practice procedures to minimise any suffering.

Project 14	Improving the Pharmacokinetics and Tissue Targeting of Antimicrobials
Key Words	antimicrobial, melioidosis, Burkholderia pseudomallei, Francisella tularensis, Pharmacokinetics
Expected duration of the project	3 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Some antimicrobials in use today, although very effective in a test tube do not persist in the body long enough or reach the correct tissues in the required concentrations to properly treat infections. Getting drugs to the brain is a particular challenge. This means that they have to be administered repeatedly to be effective or are poorly effective. Studies have demonstrated that reformulating some drugs with a carrier system can increase the length of time they spend in the body or target the drugs to specific tissues where they needed (such as the brain). The objectives of this project is to take antimicrobials and improve them by reformulating them so that they are more effective. The project will concentrate on reformulating drugs that are used or could be used to treat difficult to treat bacterial infections and viral infections (particularly those that infect the brain). These formulations could therefore be used to treat humans suffering from melioidosis *(caused by Burkholderia pseudomallei) or* tularemia (caused by *Francisella tularensis*) or encephalitic viruses such as Venezuelan Equine Encephalitis Virus (VEEV).

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)?

Potential benefits include better drug formulations for hard to treat bacterial and viral infections. This could include drug formulations that are more likely to work or drug formulations that can be given less frequently.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use approximately 1000 mice 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 levels of severity? What will happen to the animals at the end?

All experiments are expected to be mild as we are testing drugs with known toxicities (albeit in new formulations). All experiments will be of short duration (no longer than 48 hours). All animals will be humanely euthanized.

Application of the 3Rs

Replacement

The ability of the body to breakdown and eliminate a Drug is complex and is affected by multiple parameters including the route of administration, multiple cell types and organs, blood pressure and metabolism which are as yet impossible to replicate in any in vitro system. We have considered the use of invertebrates, but these have different circulatory systems and metabolism.

Reduction

We will continually review our results to adjust the number of animals in experimental groups so that we use the minimum number of animals to have statistically valid data. We will use available literature before doing a specific experiment to inform the time points that we use and therefore the number of animals.

Refinement

Mice are commonly used in these types of experiments as they have similar physiology to humans. Mice are also used by other workers who study infections, which means our results can be easily translated into infectious disease models. Mice will undergo a mild procedure for the minimum amount of time necessary to obtain results (lno more than 48 hours, but the vast majority will be considerably less).

Project 15	Molecular Regulation of Mammalian Development
Key Words	Genes , Oocyte, Development
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (a) basic research;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The overall objective of this project is to increase our understanding of the molecular processes regulating mammalian embryo development. Particularly we want to study the function of specific genes involved in this process.

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 discovery that certain factors can ‘reprogramme’ mature cell types into stem cells has holds great potential for new biomedical applications, such as cell replacement, drug testing and disease research. Reprogramming allows us to turn any cell of the body into a stem cell. However, the mechanisms involved in this technique are only just being identified and the success rate of the method remains very low. One way to improve our understanding of reprogramming is to study the natural programming mechanisms that begin after fertilisation in the mammalian embryo. In addition, basic scientific discoveries resulting from this programme of work will provide knowledge that would benefit couples undergoing assisted reproductive technologies such as in vitro fertilisation.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice Approx 2,650 over 5 years. The majority of these mice are adult mice; 2% are fetuses at later stages of gestation and neonates.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

In order to provide eggs and early embryos, female mice will be given hormones to maximise the number of eggs and embryos produced. They are then killed by a humane method and the embryos collected, usually one or three days later. During

the procedure these animals will only experience transient pain at the time of injection. Using molecular laboratory techniques we will identify and characterise the key factors involved in the regulation of gene expression during early embryo development in vitro – so thevast majority of the mice we will use are bred and killed for egg and early (up to day 4) embryo harvest and not subject to any invasive procedures. The ability to alter specific genes in the laboratory provides researchers with the opportunity to study the function of a particular gene. We will culture the eggs and embryos in specialist culture systems; assess their development and the effect of modified gene function. Very occasionally we may need to perform surgery on the mice, under general anaesthesia (for example to transplant some embryos into female mice or to perform a vasectomy on male mice). These are essential techniques (with moderate severity) but good surgical techniques, anaesthetic and pain relief will be used during and after surgery to minimise adverse effects. The mice are allowed to recover and will monitored closely post-operatively. Some mice will deliver at term and other pregnant females will humanely killed at specific time points in early gestation. At the end of the study all the mice will be humanly killed by an approved method. We will also breed genetically altered (reporter) mice (to obtain eggs and early embryos). These mice have been generated under other project licences. The effects of the genetic alterations (a fluorescent marker tagged to a protein of interest) are negligible, and the animals suffer no adverse side effects of this alteration. These mice will be superovulated and mated to obtain early embryos, experiencing no more than the same minor discomfort at the time of injection, as the control mice described above.

Application of the 3Rs

Replacement

All proposed studies build on extensive data derived primarily from *in vitro* and biochemical studies carried out either by this group or by the international scientific community. We will use mice mainly as egg/embryo donors with the remainder of the experimental work being carried out in the laboratory *in vitro*. We cannot obtain eggs or embryos without the use of live animals. We sometimes need to implant embryos into female mice to observe development in vivo because our current culture systems cannot support development beyond day 4 of development (i.e post-implantation development). In addition, reporter mice which have a fluorescent marker tagged to a particular protein of interest are an essential tool for molecular studies; allowing us to monitor and quantitate the reprogramming efficiency of our experiments in vitro.

Reduction

Experimental design is given priority with power analyses conducted prior to the research study to determine an appropriate sample size to achieve adequate statistical significance. Appropriate positive and negative control treatments are

included where necessary. All procedures are carried out by highly-trained staff using well-established protocols to optimise experimental design. The number of egg and embryo donor animals used will be minimised by giving the animals hormones to increase the number of eggs they produce, this increase the number of eggs recovered by approximately fivefold. Careful statistical treatment of all data will be undertaken, gaining as much information as possible from each experiment.

Refinement

The mouse is selected as a model species for these studies for several reasons. Firstly, there is more information available about this species than any other in genetics, molecular biology and reproduction. Secondly, the short reproduction interval allows studies to be completed more quickly than in any other mammal. Finally, more consistent observations can be expected as inbred strains are maintained in a closely controlled environment. All mice undergoing surgery will receive pain relief (analgesia) and good post-operative care.

Moreover, all of our animals are housed under pathogen free, environmentally controlled conditions. Animals are routinely monitored for the presence of pathogens that could potentially lead to infections. Our long term goal would be to replace the use of mice. However, currently there are no alternative in silico models or cell culture systems that can be used.

Project Title 16	Developmental Dynamics of Tissue Formation
Key Words	developmental biology, spinal cord, central nervous system
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes	(a) basic research;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

We study how the spinal cord and the trunk of the body is formed in embryos. Despite the complexity of embryonic development these tissues form in a remarkably reproducible and reliable manner. Our goal is to identify the genes involved in the developing spinal cord and body and determine how they work to produce and organise these tissues. The main reason for this work is to provide basic understanding of fundamental mechanisms. However, this knowledge will ultimately help develop and improve medical practice that will be beneficial for the treatment of human and animal disease and trauma.

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 work contributes to understanding how complex tissues of multicellular organisms develop in a precise and reproducible manner from initially equivalent cells. This will shed light on diseased and damaged tissue and, in turn, this will help in the development of therapies for these conditions

What types and approximate numbers of animals do you expect to use and over what period of time?

Over 5 years we will use about 12000 mice and 1000 zebrafish.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The vast majority of our regulated procedures involve breeding of genetically altered animals or minor interventions such as injections, with minimal effects. Some mutations may directly lead to mild effects on the animals, such as supernumerary toes and some procedures involve surgery. Such animals will be closely monitored,

and anaesthetics, analgesics and/or other ameliorative procedures will be used as appropriate. In all cases, animals will be humanely killed if there are signs of pain, distress or suffering above agreed limits. We are careful about group sizes, using the minimum numbers of control and experimental animals compatible with robust conclusions, making use of statistics when appropriate.

Application of the 3Rs

Replacement

We work with a several different animal species – mouse, chick embryos and zebrafish. Each species has distinct advantages with respect to the techniques and knowledge available for genetic, embryological and cell biological analyses. The complexity of embryonic development, which arises from multiple interactions between different cell types, involving short and long range signalling molecules, and complex morphogenetic events over time, requires in vivo analyses.

Reduction

We carefully design experiments to sure that we use the minimum number of animals required to give clear scientific answers. We also make extensive use of in vitro assays, in particular cell culture and in silico mathematical modelling and simulation. This greatly helps experimental design and also reduces are use of animals. We use several hundred chick embryos every year, from embryonated eggs before 2/3 of the gestation period. The accessibility of chick embryos allows us to do experiments that would otherwise have to be performed in mouse embryos and require the termination of the pregnant female. This results in a substantial reduction in the number of animals we use.

Refinement

The similarity of the spinal cord and body in all vertebrates mean that the data generated in each species is relevant to the human situation. The use of non-mammalian vertebrates, such as chick embryos and zebrafish, provides critical evolutionary insight and also reduces the use of mammals in our experiments.

Project 17	**Role of centrosomes, centrioles and cilia in vertebrate development**	
Key Words (max. 5 words)	Centrioles, cilia, vertebrate development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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)	We wish to understand how structures called cilia are involved in various diseases. Cilia are hair-like structures on the surface of cells, for example the airway where they sweep up dirt from the air we breathe in. We wish to test if these cilia are affected by the same process that causes Parkinson's disease in people. If that is the case, damage to cilia could be a contributory factor in how Parkinson's disease develops. We also wish to work out how a group of diseases that includes spina bifida are caused. We think that in these diseases, it is a combination of faulty genes that causes the disease, rather than one gene alone, which is the case in cystic fibrosis and haemophilia, for example. We will test this by generating zebrafish that carry mutations in multiple genes.	
What are the potential benefits likely to derive from this project (how science	This project will advance basic science, adding to fundamental understanding of these processes that go on in cells and the body. It will generate new	

could be advanced or humans or animals could benefit from the project)?	perspectives on two important diseases. People will benefit because this knowledge could give rise to new diagnostic tests for Parkinson's and spina bifida and maybe even new treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of the study, we will keep ten breeds of fish, about 32 fish per breed and keep two generations of each breed so 640 fish in total. These fish will live a full life — about 2-3 years — as we will be studying the eggs they lay. The eggs will be destroyed before the growing embryo can feel any pain or sensation, It is unlikely that the fish strains will experience any harm by carrying the mutations.
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 vast majority of the animals used, the adverse effects are likely to be mild or sub-threshold. We will be generating and breeding transgenic fish. They will be carriers of a mutation not sufferers. We may, in rare occasions, have to let fish that could suffer from the mutation grow to the stage where they might feel the effects but we would aim to euthanise them before this stage. This is still useful as we will be able to see changes in their organs due to the mutations they have. All the other animals, that we use for breeding only, will live a full life of 2 years and will be euthanised before they suffer from age-related diseases.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals as cells that are grown outside of an animal do not sufficiently resemble those in an animal for us to fully learn how they behave and are affected by different genes and mutations. How cells behave is strongly affected by the cells neighbouring them. Many tissues and organs are complex, finely detailed structures with many different types of cells. For the tissues we wish to study we cannot yet make these structures in a test tube so we have to use animals. Other animals, such as insects, have different arrangements of cells in some organs e.g. the brain or lack some structures e.g. the backbone so using them is not an option either.
2. Reduction	We will use well known statistical calculations to work

Explain how you will assure the use of minimum numbers of animals	out how many fish to use. In fact, our initial calculations show that the numbers of eggs we need is below the number that a viable population (to avoid inbreeding) would naturally generate. Fish need to breed regularly to prevent the females suffering the equivalent of constipation (with eggs).
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.	Zebrafish lay eggs so the fish embryos, which cannot feel anything for the first five days, develop outside of the mother. This means that we can see the effects on the embryos without doing anything to the mother or causing harm to any fish fry. The only experiment the parent fish are put through is being allowed to breed, which is a natural behaviour. We have successfully kept fish for many years. Our fish live a long time (for fish, at 2-3 years) so they are healthy, with good water quality. We have enriched their tanks so they have objects to swim around and hide between. This keeps them active and allows them to display natural behaviour. We feed them live invertebrates (shrimp, worms) so they can engage in their natural feedinci behaviour.

Project 18	Understanding gene function by phenotyping genetically altered mice
Key Words	
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Remarkably little is known about the function of many of the 22,000 or so genes in the human genome. One way to determine the function of genes with otherwise little knowledge available is to study mice with known genetic changes and examine them for characteristics that are different to normal, a process known as phenotyping. Over the next five years we seek to uncover the role of approximately 3-4% of those genes in causing or protecting against a wide range of diseases, such as cancer, obesity, diabetes, infertility, developmental, neurological and immunological disorders, and bone and cartilage disease, as well as their role in normal development. This provides a first step towards understanding the function that these genes may play in health and 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 will study the consequences of genetic changes in mice in a manner that is standardized and consistent, using tests that have the ability to be translated into human disease. We, along with others around the world, are generating this as a resource for other scientists to use. The resources and data generated by this

project will be freely available from international repositories and websites. This will allow us to build an encyclopaedia of mammalian gene function that can be used to drive forward the scientific community's understanding of health and disease. This in turn produces the long-term goal that the scientific community will be better at diagnosing disease and may identify new targets to fight disease.

What types and approximate numbers of animals do you expect to use and over what period of time?

Over the next five years we will perform experimental procedures on genetically modified embryos, new-born and adult mice, together with suitable non-genetically modified controls, for 3-4% of protein coding genes (~700-800 genes). The maximum number of animals to be used over the 5 year period of this project is 91,000.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

New lines of genetically altered mice will be studied in this project. It is not possible to predict the consequence of each genetic alteration but from our previous experience we expect only 3% to present with adverse effects. These may be managed through implementing remedial actions such as the use of extra-long water spouts for particularly small animals. Alternatively, for the very small proportion of lines with more severe adverse effects we will stop generating homozygous animals and focus our studies on the less affected but biologically important heterozygous mice. The vast majority (>97%) of the lines we study are not expected to display any adverse effects as a consequence of either the genetic alteration or the experimental procedures that allow us to characterise the mice and the in-life experience for those animals will be classed as mild. At the end of the studies, the mice will be killed using humane methods, with blood and tissues collected from them for important and informative downstream analysis such as blood cell counts, blood glucose levels and tissue imaging.

Application of the 3Rs

Replacement

Much can be gained from studies using computers and laboratory cell lines as well as study of human populations, but these yield incomplete information about the role of a gene in tissues, in development, or in an organism. To understand the role of genes in human disease, we must examine their activity in the context of other genes, other cells, other tissues and in the course of organ or embryonic development. Using model organisms, the effects of a faulty gene can be studied in great detail, defining when and where the gene acts, how it works and how it interacts with other genes.

The mouse is essential to this research because it is an excellent model for human development and disease and its genome shares the vast majority of its genes with human. Changes in genes of the mouse can closely mimic the changes seen in human disease as the mouse has the same basic tissues and organs and shares much of its physiology with humans.

Reduction

In selecting genes for study we will examine proposals from researchers and clinicians working in the research areas mentioned earlier. Wherever appropriate and possible, we will undertake computer analyses of gene activity prior to research using mice. We will also examine available databases to identify genes that have been used previously to make genetically changed mice: this is to prevent unnecessary duplication of work and consequent inappropriate use of animals.

Selected genes will be inactivated in stem cells and mice derived from these modified stem cells. Mice will be mated to derive the desired genetic features. For each mouse line, primary data will be generated from the statistically determined minimum number of animals using a limited set of mild tests covering aspects such as growth rate, cancer predisposition, fat and skeletal components and blood composition. 'Positive' data (indication of a disease/development related link) as well as 'negative' data (indication that there is no link) will be freely available for the whole scientific community to access.

Each mouse line will be preserved by freezing embryos or sperm, which will eliminate unnecessary stock maintenance and allow for distribution. By making our developed lines openly available to other licensed researchers we hope to eliminate the need for others to generate these genetically altered mouse lines, and so reduce duplication and, ultimately, numbers of animals used for research. By making the entire resources of the project openly available, it is expected that many research programmes will be advanced.

Refinement

We will seek to minimize suffering by ensuring that only mice required for experimental purposes are requested. We are constantly refining our experimental processes to minimise harm and reduce adverse effects on the mice without affecting the experimental data. This includes steps such as lifting our animals using cardboard tunnels rather than the base of their tail, and using gaseous anaesthetic from which the animals recover in a matter of minutes, rather than injecting anaesthetic agents into their blood stream which takes them longer to wake up from. We have also chosen a protocol for monitoring blood glucose levels where mice are usually fasted for 4 hours rather than overnight as with other protocols. For studies where the data suggests that newborn pups are affected, we put in place routines to minimize stress levels on the mother, such as only removing part of her litter at a time and limiting the duration that pups can be removed for.

In addition, we are using tests that are similar to those performed on human patients, scaled down for the mouse, which should provide more translation and better use and reliability of the data. The tests themselves are well established and have been optimised for generating data suitable for coming to scientific and statistical conclusions.

Project 19	Production and Maintenance of Genetically Altered Mice
Key Words	Production, Geneticall Altered, Archiving
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The production of genetically modified animals to unravel the functional role of genes employs a variety of model organisms amenable to gene manipulation. Incredible advances in the associated technologies over the last 20 years have enabled many subtle and controllable genetic manipulations of the mouse genome. The mouse itself is essential to our research because, as a mammal, it shares many of the developmental milestones and disease states that we, as humans, will experience in our lifetimes. Changes in the genes of the mouse can closely mimic the changes seen in human disease as the mouse has the same basic tissues and organs, and shares much of its physiology with humans. The knowledge we gain from studying this model organism helps us understand the role of genes in human disease, and allows us to examine gene activity in the context of other genes, other cells, other tissues and in the course of development. Therefore, through the use of this model organism, the effects of any faulty gene can be studied in great detail, defining when and where the gene acts, how it works and how it interacts with other genes.

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 approach to mouse production is to look for the most efficient use of all our resources. All models that are created are aligned to the goals and aspirations of the other projects this licence will support. The expertise available to us and the application of well-developed strategies ensures high quality mouse models with known and, where possible, standardised mutations. With our experience in

production and breeding techniques, this licence seeks to provide a service to other related licences, such that they can take full advantage of this highly efficient and refined production capability, thus minimising animal usage at this stage of their own projects. These efficiencies are used to further inform others and we actively share our knowledge and resources with the scientific community to allow them to take advantage of the production processes we have optimised. For example, selected genes will be manipulated in genetically defined mouse stem cells. As the manipulated gene is then established within the genome of a living mouse, early matings will be used to give a primary review of any observable features of the mouse line. A standardised breeding and early welfare screen is then employed by dedicated teams, allowing them to rapidly review breeding and welfare strategies to ensure the minimal number of mice are required to establish a genetically altered mouse model.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mouse 450,000 animals predominantly for breeding and production of GA mice. The vast majority of these mice (>90%) are expected to remain below the mild severity limit. 5 years

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

From our experience the vast majority of animals will experience no adverse effects due to the primary purpose being the breeding and maintenance of such models. A small percentage (up to 6%) will be animals that have a novel gene inserted into their own genome. Of these animals less than 1% will present with either a mild (equivalent to a medical injection and highly transitory in its nature) or a moderate phenotype (causing prolonged effects within the animals, but where husbandry or environmental alterations will alleviate these effects). Any animal that experiences adverse effects that cannot be ameliorated, will be killed humanely and in a timely manner. All animals that have reached the end of their study will be killed using a humane method of killing. Any animal that experiences adverse effects that cannot be ameliorated will be killed humanely and in a timely manner. All animals that have reached the end of their study will be killed using a humane method of killing.

Application of the 3Rs

Replacement

Extensive *ex vivo* analysis is integral to all at our Establishment and is always the first option considered when new biological areas of interest are identified. However to study the full effect of a gene mutation it is essential to study it in context with all molecular, developmental and physiological interactions provided within a living mammalian system. Due to its similarity and availability of extensive genetic

manipulation techniques, the mouse is ideally suited to allow us to study these interactions.

Reduction

We continuously look at ways to minimise the number of animals used to propagate mutant models. Embryo numbers and recipients are carefully aligned to the number of clones required, while efficiency rates are routinely monitored ensuring the fewest number of animals are required to obtain the required number of new mutant models. Archiving has moved to using sperm as the predominant method of cryopreservation, again reducing the number of animals required to secure a line.

Efficient colony management ensures that only colonies that are actively required are mated and produce animals. Those that are no longer required are cryopreserved and closed at the earliest opportunity.

We have calculated that each colony we produce requires 300 mice to reach a stable colony. By distributing and archiving in sustainable archive we have been able to potentially reduce the global production by around 750,000 animals should all colonies be recovered and progressed.

Further by the use of CRISPR/Cas9 during production we will save around 100 mice per colony. This will allow us to reduce the number of animals we generate by around 11,000 per year.

Refinement

Comparative anatomical, embryological and physiological studies have shown that mice and humans have the same basic organ systems, skeleton and reproductive cycles. These similarities, coupled with the rapid advances in technologies available to manipulate the mouse genome, make the mouse the most suitable model to mimic human disease condition.

Mice are only created if they are required for experimental analysis in line with the programs of the establishment. Careful monitoring and adaptation of our processes has led to refinement of the stages of the processes required for the maintenance and provision for experimental purposes. This has seen us reduce the average cage holding within the facility from 19 to 13 per colony showing elements of reduction driven by refinement. This has been underpinned by the development of software that allows us to have greater oversight on the operation and requirements of large scale production. This is now being made available to other establishments to allow them to also gain such benefits.

On-going review of breeding and production data coupled with standardised welfare observations, allow us to further refine procedural, production and breeding protocols both within this licence and in the provision of optimal breeding strategies for other project licences at the establishment.

Project 20	Caveolae and endothelial transcytosis
Key Words	Caveolae, endothelium, blood vessel, endocytosis
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (a) basic research;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Caveolae are small folds in the surface membrane of many mammalian cell types. They are especially abundant in the cells that form blood vessels, and in fat cells. While we have made recent progress in understanding the molecules which provide caveolae and how these molecules assemble together, the cellular functions of caveolae are not so clear. This project aims to define the physiological functions of caveolae, particularly in blood vessels.

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 already know from the health problems of people with very rare genetic problems leading to lack of caveolae, and from the effects of artificially removing genes for making caveolae in mice, that caveolae are important regulators of the permeability of blood vessels. This is a process of fundamental physiological and medical importance, and is relevant in many disease states. In addition, the cells forming blood vessels in the brain block access of many drugs to brain cells, and understanding mechanisms by which blood vessel permeability is controlled is important and may lead to better access for drugs to the brain.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice, 17,000, 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

We do not expect any of our procedures or experiments to cause more than temporary discomfort to mice. All mice will be cared for by a veterinary surgeon and

skilled technicians, and invasive procedures carried out under anaesthesia. At the end of experiments mice will be humanely killed.

Application of the 3Rs

Replacement

Blood vessels are unique to vertebrates and experiments in mice can provide information directly relevant to human physiology.

Reduction

Mouse breeding will be carefully managed to ensure excess animals are not generated. When we carry out experiments the minimum number of mice needed to generate robust, statistically significant results will be used. We aim to develop new types of experiment where transport across blood vessels is visualised directly, which should yield relevant information with relatively small numbers of animals.

Refinement

Mice are well established as the model system of choice for studying mammalian physiology, as it is relatively easy to make genetic changes to mice and ask what are the consequences of these changes. It is not possible to use non-vertebrate models to study blood vessels. All well established procedures for making genetic changes in mice will be carried out by highly skilled technicians and are constantly refined to minimise suffering. When new experiments are introduced pilot experiments with small numbers of animals will check for adverse effects.

Project 21	Deciphering the mechanisms of early embryonic development
Key Words	Vertebrate development, stem cells, Xenopus
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (a) basic research;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

To obtain fundamental understanding of embryonic development in vertebrates, particularly the events that occur immediately after fertilisation.

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 work will improve basic understanding of developmental mechanisms; it may help reduce developmental abnormalities in human beings; and it will help in the directed differentiation of stem cells for future clinical intervention.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use, over the course of 5 years, the following number of animals: Xenopus laevis wild type: 500 Xenopus laevis GA: 150 Xenopus tropicalis wild-type: 3000 Xenopus tropicalis GA: 2000 Danio rerio (Zebrafish) GA: 5000 Mice GA: 1000

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Adverse effects are few. The level of severity will be mild. Xenopus species and Danio rerio will be re-used or subjected to Schedule 1 killing. Phenotypes of GA animals are expected to be mild.

Application of the 3Rs

Replacement

Our research is aimed at understanding the regulatory mechanisms that initiates zygotic transcription and determining how these new genes then go on and control

early embryo development. There is no alternative to using animals for these experiments: *ex vivo* systems such as tissue culture or organ culture cannot recapitulate the complexity of the early embryo.

Reduction

Most of our work involves the use of eggs or embryos of *Xenopus laevis* and *Xenopus tropicalis* collected from adult females injected with gonadotrophins. Because adult females lay thousands of eggs in a single spawning, we are able to share the eggs amongst members of the lab, maximising the output per procedure and therefore reducing unnecessary waste. In addition, the large numbers of eggs and embryos afford us with the additional ability to increase the statistical power of our analyses due to the inherently large sample (N) sizes. Since we can generate embryos from different females on the same day we can collect biological replicates under identical conditions and process them at the same time thereby limiting sources of variation in our experiments.

We shall also reduce our use of animals by establishing *in vitro* organoid culture systems that can replicate in some instances in vivo cellular niches.

Refinement

Xenopus species and zebrafish are invaluable and powerful model organisms to study early vertebrate development. Their large eggs, laid in large numbers, are amenable to the analysis of the first gene regulatory pathways initiated following fertilization. *Xenopus* and zebrafish genomes are sequenced and are flexible tools for genetic studies including the use of high throughput genomic sequencing technologies (e.g., RNA-seq, ChIP-seq). This flexibility will be central to understanding the mechanisms of early vertebrate development. Collection of eggs from frogs and fish is a mild procedure and we use embryos prior to independent feeding.

Insights from the fish and frog will be confirmed in the mouse, the closest mammalian model to humans. Here we shall assess developmental events using embryos from WT or GA mice..Further studies will use organotypic systems and tissues will be harvested from adults killed by Schedule 1. Phenotypes of GA mice can be variable and we will perform extensive welfare assessments to characterise phenotypes and to ensure that any imported and newly derived lines are appropriately housed and provided with specialist care if needed.

Project 22	**Fungal infection, diagnosis and therapy**	
Key Words (max. 5 words)	Fungus, infection, antifungal, therapy, diagnosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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
	x	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 are to investigate how fungi cause infection, including involvement of specific host and fungal factors, and to evaluate new drug treatments for fungal infection.	
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)?	Fungal infection affects around one fifth of the world's population at any single moment in time, causing skin, nail, vaginal, oral and bloodstream infections. These latter infections are occur mainly in severely ill patients and are difficult to diagnose, with only limited treatment options available. Understanding how fungi cause disease could identify potential molecules which could be used to design new diagnostic tools, and our role in evaluating new antifungal drugs could accelerate the development of new drugs for clinical use.	
What species and approximate numbers of animals do you expect to use	We expect to use the following species and numbers over the next 5 years: Species: mouse 9700 (includes 3000 GA animals),	

over what period of time?	100 rats, 80 guinea pigs and 50 rabbits. These are approximate numbers based upon our prior experience, currently funded grants, future grant applications and research plans. The majority of animals used will be mice, but small numbers of rats, guinea pigs and rabbits may be used under specific circumstances, e.g. requirement for repeated blood sampling or a requirement for larger tissue samples for downstream processing.
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 will be used to model fungal infection, with fungi administered via different routes depending upon the infection to be modelled. Some infection models require manipulation of the host, either through maintenance of oestrus (through surgery or oestrogen injection) or through immunosuppression. The host may also undergo blood sampling or imaging. Infected animals will be used to elucidate the role of fungal virulence factors on disease, to evaluate the efficacy of new drugs or new treatment regimens, or to investigate the role of the host immune system or prior infection on susceptibility to fungal disease. Through refinement of our models and monitoring procedures, the vast majority of our animals are classified as mild or moderate, with less than 3% of animals estimated to potentially be classified as severe. All animals will be humanely killed and sampled to provide additional infection-related data at the end of the experiment, enhancing the information gained from each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Infection is the result of the ability of a pathogen to cause disease and the inability of a host to detect and fight disease due to alterations in the host immune system. Although we can model some steps in infection using laboratory-based model systems, such as fungal interaction with renal epithelial cells, which models the initial steps occurring in the kidney (we cannot yet fully model infection progression nor can

	we fully evaluate antifungal efficacy in the host without the use of animals. We will continue to monitor the literature for new laboratory models applicable to our area of research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use minimal numbers of animals in our work through careful planning of experiments and using the minimal number of animals in treatments and control groups, but which still allow detection of biologically relevant differences. We routinely use Power analyses to determine the minimum number of animals required for any experiment. We will continue to develop and evaluate new techniques to allow us to follow infection in individual animals, rather than sampling groups of animals at each of the time points of interest.
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 majority of our work uses well-characterised fungal infection models, which we have been working with for over 10 years. The majority of these models are based in mice, with other species used only when larger biological samples are required as part of the study. We have already refined our models by significantly reducing the length of infection (3 days compared to 28 days). New models will be developed through pilot studies using small numbers of animals. We will minimise harm to animals by puffing in place clear and careful monitoring systems. Anaesthesia and pain relief will also be administered where appropriate and procedures will only be carried out by highly trained and competent personal licence holders (trained postdoctoral scientists and PhD students from my research group and the technicians based in the animal facility).

Project 23	Supply of Biospecimens from Rats and Mice
Key Words	Blood, Tissues, Drug discovery/development
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

To use animals to provide blood and tissues to enable experiments to generate data to support the development of effective and safe medicines to treat diseases where there is currently a clinical unmet need e.g., cancer and diabetes

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)?

Contribute invaluable scientific data to support and progress potential new medicines where there is currently an unmet clinical need. Conducting investigations using blood and/or tissues taken from animals reduces the number of potential new medicines requiring evaluation in living animals and can be used to establish whether conducting experiments on living animals would be beneficial.

What types and approximate numbers of animals do you expect to use and over what period of time?

Rats: 500 over 5 years Mice: 750 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 levels of severity? What will happen to the animals at the end?

All the animals used under this licence will be general anaesthesia will be kept under general anaesthesia throughout the procedures conducted and will not be brought back to consciousness. They will be humanely killed while still under anaesthesia

with an overdose of anaesthetic. Therefore animals are not expected nor likely to experience any adverse effects.

Application of the 3Rs

Replacement

In drug discovery, research programmes rely, in part, on biological materials obtained from human or animal sources to validate and confirm disease- associated pharmacological targets and mechanisms for potential new medicines.

This programme of work supports the replacement of using living animals by enabling the supply of high quality biospecimens (blood and tissues) where primary cells are needed for experiments due to lack of appropriate cells from existing sources or instances where it is not possible to use cell culture techniques.

There are a number of promising technologies in development which aim to utilise human cells to recreate the physiological functions of organs without using animals. However, these approaches do not offer an alternative to replace blood, blood products, body fluids and tissues in a suitable form for use in in vitro investigations required to support the research and development of new medicines at the present time.

Reduction

The number of animals chosen to supply any single request is minimised by using collection techniques likely to succeed, including taking blood under non-recovery anaesthesia to ensure that a large volume, non-clotted samples can be obtained, negating the need to use more animals than required.

Biospecimen requests will be co-ordinated in order to supply a number of biospecimens from one animal (e.g. whole blood, pancreas, femurs and liver) to a number of requesters for their individual purposes. This should result in improved efficiency of use of animals (i.e. reduce the total number used).

Refinement

Where there is scientific need to preserve tissue integrity/architecture or obtain high volume and quality blood sample then taking samples under appropriate and well maintained non-recovery anaesthesia is considered the most refined approach.

Personal licensees taking samples will be well trained in the techniques involved to ensure high quality samples are obtained quickly, effectively and with minimal impact on animal welfare.

Project 24	Preclinical Imaging in Biomedical Research
Key Words	PET, imaging, drug
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The objective of this work is to provide unique data in rodents, (i) to support the development of tool radiolabelled compounds for the medical imaging technology, position emission tomography (PET), (ii) to characterise novel drugs and therapies using imaging; and (iii) to use imaging to provide key knowledge of healthy and disease states within the living body.

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 is expected to optimise clinical imaging studies in several ways but particularly by the development of tool compounds for PET. The development of drugs by our collaborators and customers including both Pharma and biotech companies, will be facilitated, thereby reducing attrition. In addition, using imaging and related techniques as an important tool, the work will contribute new fundamental scientific knowledge on normal and disease processes within the body.

What types and approximate numbers of animals do you expect to use and over what period of time?

Approximately 700 rodents (rats, mice, Guinea pigs & gerbils) may be used per year.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Where possible, normal free-living rodents will be used under this project. Animal models of disease such as Parkinson's Disease, respiratory disease, rheumatoid arthritis and cancer may be generated and used, where the experimental question may not be answered using normal animals. Aseptic techniques will be used for surgical preparation of recovery animals, together with antibiotic/analgesic cover, where appropriate. Possible toxic effects of compound administration are expected to be rare and mild as the lowest possible dose possible to give scientific valid data will be used. The body temperature and other physiological parameters of the animals will be monitored throughout the procedures where possible. If adverse effects were to occur then suitable action based upon the best care practice and/or veterinary advice will be taken with immediate effect. Typically, studies will involve an imaging technique to provide quantitative in vivo data on a compound or biological process. Alternatively, a simple dosing and sampling regimen may be employed. Blood samples and/or tissue samples will be collected when required. By analysing the data, the concentration of radioactivity in the tissues will be determined and biological parameters generated. The majority (>95%) of the animals are not expected to show any signs of adverse effects that impact materially on their general wellbeing. No more than 5% of animals are expected to show clinical signs of a moderate severity. The animals will be humanely killed at the end of the procedure.

Application of the 3Rs

Replacement

New drugs and PET tool compounds are required. Studies using tissues and cells or computer simulation are typically used to investigate the characteristics of compounds and/or their targets of interest. However, as these drugs and PET tool compounds are being developed for administration in humans, their effects on the body as a whole and the effect of the body on the drugs themselves are important to know. Therefore they need to be investigated in live animals. In addition, some biological processes may only be investigated in live animals

Reduction

For each study, the minimum number of animals required to obtain scientifically valid data will be used. Imaging enables the characteristics of a compound or a process within the body to be measured over time, negating the use of a larger number of

animals killed at discrete time points and will thus reduce the number of animals that would otherwise be used.

Refinement

Rodents, commonly rats and mice, will be used under this project as the lowest sentient species that may be used to produce satisfactory results. There is a considerable amount of historical data using these species for imaging studies. Opportunities for experimental refinement will be sought throughout the project. Good, sympathetic handling techniques will be used to minimise discomfort to the animals. To reduce distress, single housing will be reduced to a minimum and additional enrichment will be provided wherever possible.

Project 25	Understanding mechanisms of fibrosis
Key Words	Fibrosis, scar, myofibroblasts, therapy, diagnosis
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Fibrotic diseases are increasing and a major cause of morbidity and mortality worldwide. In some cases, end-stage diseases can be treated by transplantation; however, there is a huge shortage of donor organs; significant side-effects from immunosuppression; and focus on end-stage disease is too late. Urgent development of novel diagnostics to determine stage of disease and anti-fibrotic therapies are needed. This requires a better understanding of the underlying mechanisms of fibrosis to develop hypothesis based approaches to identify novel dynamic markers of disease and targeted strategies for therapeutic intervention. The aim of this project is to provide a greater understanding of the molecular mechanisms underlying chronic fibrotic diseases to instruct identification of novel diagnostic and therapeutic targets that can be used for patient benefit.

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)?

Fibrosis is a common step in the progression of the majority of chronic diseases. However, there are no approved anti-fibrotic drugs and diagnosis remains poor. Our work in this area has already uncovered novel mechanisms implicated in broad organ fibrosis that are currently under discussion with pharmaceutical companies as

novel diagnostic / therapeutic strategies in fibrosis. There are clear implications for patient benefit and this has only been achieved by proof of principle using both in vitro and in vivo models of disease.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use rat but more often mouse, particularly because of the ability to use genetically modified strains. Over a period of 5 years, with funding and staff / students working on these projects, I would expect breeding numbers to reach approximately 10,000 mice using several different genetic strains and for experimental protocols ~1,500 rats and ~18,500 mice (a mix of wild type background and genetically modified animals drawn from those bred under this licence or other appropriate licences). Where possible we will try to use both sexes from our transgenic breeding, but this may not be appropriate as females can be resistant to developing liver fibrosis.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

In most instances, tissue from animals will be removed for studies in the laboratory. In some instances, animals will be treated with agents that cause fibrosis. Although transient discomfort may occur at the time of administration the animals appear normal soon afterwards. Similar to humans, animals can sustain fibrotic injury for a long period of time with no apparent symptoms. In the rare scenario that an animal shows signs of organ failure the animal will be put down to ensure the animal does not exceed the severity limits set out in the project. Some animals will undergo surgery to induce fibrosis, but these are not life-threatening procedures. Animals will suffer moderate adverse effects from this, which are similar to and primarily associated with the surgical procedure, the effects of which will be alleviated with pain-killing drugs.

Application of the 3Rs

Replacement

Despite progress in understanding the biology of fibrotic diseases, these discoveries have been unsuccessfully translated into the clinic. Fibrotic diseases are complex which develop and resolve over many weeks; involving the organ, immune system and cell-cell interactions. For this reason, it is not possible to study these events in isolation in an *in vitro* / ex vivo system.

Reduction

Power calculations performed based on an important component of fibrosis (collagen deposition) indicate 6 animals per group are required to analyse the fibrotic processes. For example our experience of biological variability shows fibrotic livers

of 6 weeks CCl_4 treated rats have a mean collagen (hydroxyproline) content of 1.45 ± 0.25 (SD) mmol/g liver. Based on these data, accepting an 80% chance of detecting this difference at the level of p≥0.05, gives a sample size of $16/(1.74)^2$= 5.3 animals per group.

Where possible we will make use of archived material and importantly make use of human cells and tissue to reduce animal use.

Through refining our technical skills, see below, we are also able to reduce animal numbers.

Animal breeding will take into account the power calculations required for the experimental protocols.

Refinement

In the case of cellular studies, particularly for liver fibrosis, we will use rats as this allows a greater analysis of the mechanisms associated with the disease process compared to mouse. However, for in vivo studies, mice will be necessary based on the use of genetically modified strains.

To investigate the therapeutic potential of our findings in fibrotic disease in different organs from multiple etiologies, it is necessary to use more than one model of injury. We have chosen established models of organ fibrosis that have good comparison with human disease and have been refined over many years in labs worldwide.

As evidence of limiting animal experimentation through refining our models, improved technical skills and post-operative care we have reduced the mortality of bile duct ligation from 30% to ~10% on our current liver fibrosis models. We will ensure similar refinement in all protocols (which are much less severe).

As further refinement, and in agreement with our resident statistician, we will seek additional statistical assistance as required to refine experiments.