Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 29

Projects with a primary purpose of: Basic Research – Other research

Project Titles and keywords

1. **Fish behaviour modification through gear changes**
    - Fish behaviour, selectivity, gear modification

2. **Mammary gland morphogenesis and function**
    - Mammary, breast, integrin, adhesion, polarity

3. **Evolution and ecology of host-parasite interactions**
    - Malaria, transmission, disease ecology, life history strategy, phenotypic plasticity

4. **Acute and persistent bacterial disease**
    - Salmonella, Escherichia, Crohn's, Inflammation, Apoptosis

5. **Molecular probes for plant cell walls**
    - Antibodies, cell walls, polysaccharides, pectin

6. **Combating tapeworm parasitism using new model systems**
    - Tapeworms, parasitism, laboratory culture

7. **Mitochondrial biology of trypanosomes**
    - Trypanosomes, sleeping sickness, drug discovery

8. **Novel types of gene regulation in development**
    - Novel, types, gene, regulation, development

9. **Developmental Biology and Evolution of cavefish**
    - Tetra, development, evolution, adaptation

10. **Rodent Models of Dementia: Cognition and Therapy**
    - Memory, Dementia, Therapy, Downs Syndrome

<table>
<tr><td>Project 1</td><td>Fish behaviour modification through gear changes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish behaviour, selectivity, gear modification</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the project is to investigate how fish behaviour can be used to make commercial fishing gear more selective through tank experiments. Selectivity of fishing gear is particularly important in fisheries with a mix of species such as the North Sea. Therefore it is vital fishermen are selective in what they catch by retaining only the species they want to land and species within the regulated landing sizes.

A simulation of a towed gear with modifications to different components will be towed around an annular tank using a rotating gantry arm. By simulating fishing gear it is hoped we will then be able to understand fish behaviour in relation to the gear modifications and utilise this information. How the information is used depends on the reactions observed, but the purpose would be to stimulate a behavioural response from the important commercial species that will promote their capture.

One example of a potential gear modification is the use of lights to encourage or deter the fish to pass through the meshes of the net. If it is found that</td></tr>
</table>

	haddock are attracted to the lights because it makes an escape route from the net more visible, then we could say that it improves sustainability by retaining larger individuals who cannot escape through the meshes due to size but encourages the smaller individuals to escape by providing a visible exit. When fish are feeling threatened they will always choose the easiest option, if swimming along inside the net is easier than attempting to swim through the meshes they will not attempt to make an escape attempt. In the past other studies have shown certain species of fish are attracted to lights and other species try to avoid it. Therefore we will be looking to observe fish behaviour in response to lights and understand if this can be used to make the net more selective when capturing fish. Further work will observe the effects of other gear modifications on a range of commercially important fish species.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this work are that it may assist commercial fishing vessels to fish in a more sustainable and environmentally friendly way. It could potentially reduce discards which would nicely compliment the upcoming EU discard ban which will be fully implemented by 2019. It could also exploit species specific behaviour to enhance selectivity of trawls which is particularly useful in a mixed fishery such as the North sea. This would also compliment the already established schemes which are in place to help recover depleted cod stocks.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using a range of marine fish throughout the work. Approximately 1000 fish a year 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?	The expected adverse effects will come from when the fish require being tagged/marked and the act or chasing them around a tank with the simulated fishing gear. Anaesthesia will be used during the tagging procedure and it will be carried out by skilled and experienced staff. During the chasing of the fish any fish that are unable to maintain swimming speed on a regular basis due to fatigue will be removed from the experiment before they are run to exhaustion. All procedural animals will be killed by a humane

	schedule 1 approved method upon completion of a study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As we will be observing fish behaviour we have to use actual fish to learn what their responses will be to the gear modifications. A computer model would require prior knowledge of how the fish will react and is therefore not currently an option for our study but we will use the data to develop one in the longer term if possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure we use the minimum number of animals we will carefully plan our experimental design to ensure it is statistically valid with assistance from our own statistician. We will ensure we have enough fish to make reasonable conclusions from our work and have enough power to detect any changes. We will also be carrying out pilot studies which will give us an insight into how to best proceed with this licensed work.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will be using marine fish species of commercial interest to help us meet the aims and objectives of the project. It is crucial we use these species because understanding their behaviour is key to the success of this work and to improving the sustainability of these commercially important fish stocks. . We need to replicate commercial fishing as closely as possible in a laboratory environment to be able to draw reasonable conclusions from the work. By starting with observations and pilot studies we will be able to optimise the design of the study to achieve our aims in a timely manner with the least amount of animals being used as possible. We will be using encouragement rather than punishment through a food reward which is positive non-painful encouragement. Where tagging of the fish is required the method chosen shall be the least severe for the duration of the study and where possible not done at all.

Project 2	**Mammary gland morphogenesis and function**	
Key Words (max. 5 words)	Mammary, breast, integrin, adhesion, polarity	
Expected duration of the project (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)	Our research focuses on the adhesion of cells to their local environment within tissues. We are interested in how adhesion controls tissue organisation and cell behaviour in the mammary gland, and how this becomes altered in diseases including breast cancer. The objective of the project is to determine mechanisms by which the adhesion of cells to an extracellular scaffold of proteins, called the extracellular matrix, regulates cell behaviour in the mammary gland. We will characterise the role of key molecular components of adhesion signalling pathways, e.g. extracellular matrix proteins, extracellular matrix receptors on the surface of cells, called integrins, and integrin-signalling proteins, in the control of mammary gland development, function, and neoplasia. The importance of this work is that understanding how adhesion-related proteins determine cell behaviour in mammary tissue will ultimately lead to better strategies for breast cancer prediction and novel targets for breast cancer intervention. Examples of specific current projects include: The role of adhesion signalling in mammary development and function. We will determine how integrins and integrin signalling proteins, control cell behaviour and organisation within mammary tissues	

	and how they affect the local tissue microenvironment, including inflammatory responses from immune cells.
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 provide a greater knowledge of basic processes involved with the normal development of glandular epithelium, a greater insight into the causes of breast cancer formation, and may ultimately lead to the identification of new biomarkers for detecting cancers and possibly molecular targets that would be useful to target therapeutically. This work will have significant mechanistic implications for many scientists studying the role of adhesion, because what we find out using the mammary gland is likely to represent universal principals for other epithelial cells and tissues. In addition, experimental evidence obtained using *ex-vivo* cultures that altered adhesion-signalling proteins perturb tissue organisation will have wider implications for stem and regenerative medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use ~10,600 mice 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 effects and the likely/expected level of severity? What will happen to the animals at the end?	*Protocol 1*. Breeding and maintenance of genetically modified mice. Mild. No phenotypes or adverse reactions are expected in tissues other than mammary gland. Any defects arising within the mammary gland are unlikely to cause disease or pathogenic mastitis, although they may cause cessation of milk production or mild inflammation. Animals will be killed by a Schedule 1 method at the designated establishment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*Protocol 1*. Developmental processes occur in tissues in living organisms. In order to study how the protein products of genes work in developmental processes, it is necessary to alter the function of genes, which is done through transgenic means. Alternatives to studying true developmental processes are not fully possible ex vivo because development occurs in the context of multiple cells within organs, an endocrine milieu and immune responses which cannot yet be fully recreated in culture models. For the *ex-vivo* work; No established cell culture models exist which can recapitulate both mammary epithelial cell compartments, or polarize and

	differentiate as efficiently as primary mammary epithelia isolated from mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully considered all the aspects of mouse colony management and optimized experimental design to minimize the number of mice to use in order for us to: i) maintain stocks of various mouse strains, and provide female mice whose mammary glands will be used for *ex vivo* studies and ii) for genetic analysis of mammary gland development.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The study of mammary gland function requires the use of mammals. In all protocols, mice are the most appropriate species for genetic analyses using conditional-null gene deletion. In order for the new proposal to yield meaningful conclusions, we need to compare our future data with those results obtained and published in the past. *Protocol 1*. We do not expect that these mice will show harmful or abnormal phenotype during the course of their housing, because the use of mammary-specific promoters will ensure that resultant phenotypes only occur in of post-pregnant female offspring. Pilot study to establish conditional targeting to basal cells will only be performed in female mice carrying reporter genes with no gene ablation. The use of inducible promoters will ensure that resultant phenotypes only occur in females treated with gene-inducing drugs. We do not expect these mice to show harmful phenotypes. Some genetic deletion studies will be conditional and only performed in tissue culture using cells isolated from mice so that no harmful phenotypes manifest within mice. Mice will be checked routinely for the appearance of skin defects or general malaise (BCS guidelines). We will sacrifice mice if we determine that mice have health related issues that warrant immediate attention.

Project 3	**Evolution and ecology of host-parasite interactions**	
Key Words (max. 5 words)	Malaria, transmission, disease ecology, life history strategy, phenotypic plasticity	
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)	We investigate the strategies that parasites, such as malaria parasites, have evolved to maximize their fitness in terms of their ability to survive in the host they infect and to transmit between hosts. Our work involves assessing how parasites interact with each other and cope with the changing environments they experience inside hosts and the vectors, such as mosquitoes, that transmit them.	
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 research focuses on malaria (Plasmodium) parasites, which cause some of the most serious infectious diseases of humans, livestock, companion animals and wildlife all over the world. These parasites remain ahead of biomedical science, despite extensive research into their immunology, cell and molecular biology. Few new treatments or control measures have been discovered in the last decade. A different approach, using a whole-organism (evolutionary) perspective to understand how parasites behave has been neglected but is increasingly being recognised as central to advance disease control. Using an evolutionary approach provides a unique opportunity to understand parasite behaviours at all levels - from genes, to behaviour, to	

	population patterns. Malaria parasites have high medical importance and the development of new control strategies requires a better understanding of their biology. We will contribute to this understanding and the development of control/treatment strategies. These benefits will be realized by publishing in high quality, internationally recognised scientific journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Our research involves malaria parasites that infect rodents in nature. Our research questions for the next 5 years are aimed at understanding basic biology and we combine lab, theory and field experiments to achieve this. In the lab, we primarily investigate infections in mice from various lab strains (13000). We also occasionally require rats (100) to maintain our mosquito colony and we plan some small-scale experiments using voles
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?	Our experiments require monitoring parasites and hosts during infections and examining parasite behaviours, physiology and genetics. Infected animals are monitored at least once a day and in most experiments, they do not show any clinical symptoms. When infections need to be monitored throughout their natural course, animals experience weight loss and anaemia, and almost all recover fully. We monitor animals closely and euthanize any that are at risk of not making a full recovery. We take small blood samples to collect our data and to monitor the health of animals. To fulfil our objectives, most of the animals will experience symptoms with mild severity (>50%), but some moderate, and a minority severe (<10%). All animals are euthanized at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand how parasites and hosts interact and their coevolution, both parties are required. Ethically, it is not appropriate to use human malaria. Therefore, experiments are only possible with animal models. In vitro methods cannot capture the complexity and biological context of real infections. We employ mathematical modeling approaches where possible to form and refine hypotheses but ultimately, we need to use animals to formally test our scientific hypotheses.
2. Reduction Explain how you will assure	We continually harness new technical advances from the fields of evolutionary, molecular and cell biology

the use of minimum numbers of animals	and immunology to reduce numbers. This includes in vitro methods that reduce the number of animals required and shorten the duration of infections. Compared to our current project, we forecast that 15% fewer animals will be required for this project. We also use the most powerful statistical analysis methods available for data analysis as this maximises the amount and quality of information obtained from each animal. We use our data to answer multiple research questions, reducing the need for multiple experiments. Parasites are stored in liquid nitrogen, reducing the number of animals needed to maintain them.
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.	Over the last decade our work has been refined and now, the majority of animals will be euthanized before showing clinical disease symptoms. For example, we have improved the ways that we administer drugs and blood sample infected animals. We also continually refining our endpoints as we gain more experience with different host-parasite strain/species combinations and experimental perturbations. Infected animals are monitored daily. During periods when animals show symptoms of malaria they are closely monitored, including checking them every few hours, so that measures can be taken to facilitate their recovery.

Project 4	**Acute and persistent bacterial disease**	
Key Words (max. 5 words)	Salmonella Escherichia Crohn's Inflammation Apoptosis	
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)	This project aims to understand the role of cell death in infectious disease. Acute and persistent bacterial pathogens use alternative strategies to either initiate or inhibit cell death resulting in bacterial dissemination in the case of acute infection, or long term intracellular survival in the case of persistent infection. By continuing to study the pathways we have identified as being key to this axis of acute and persistent infection we aim to identify key host-pathogen interactions at the cellular level that facilitate infection. The longer term goal is to identify targets within these host-pathogen interactions that can be subjected to therapeutic intervention.	
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)?	Understanding how infection takes hold and how bacteria persist are still surprisingly not well understood. Our work to decipher the means by which bacterial pathogens undermine crucial host pathogens will advance our basic knowledge of infection and identify pathways that are not functioning properly or being undermined during infection. This will create new opportunities for creating intervention strategies.	
What species and approximate numbers of	We intend to use mice for all this work which will be carried out over 5 years. We anticipate using	

animals do you expect to use over what period of time?	approximately 1800 animals over the duration of this license.
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?	Mice will be infected with strains of bacteria that cause short term acute infections or longer term persistent infections. The severity of these techniques in moderate and at the end point the animals will be culled and tissue harvested. Adverse effects mainly are due to administration of bacteria or antibiotics and the potential for these adverse effects to occur will be minimised by all techniques being carried out by fully trained staff. All animals will be regularly monitored during infection.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cell death during bacterial infection is a highly regulated and complex process influenced by both intracellular and external signals. This signalling includes cell to cell communication between a variety of immune cells during the immune response to infection. Replication of these complex interactions *in vitro* is presently not possible. In addition the available cell lines for *in vitro* modelling are immortalized meaning they have genetic defects in cell death pathways that enable them to survive indefinitely, thus meaning they are inappropriate for use in these studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our work is carried out where possible *in vitro* to try to obtain an understanding of the processes involved in bacterial or host survival post infection. This data is then used to shape any decisions based on proceeding with *in vivo* work. Previous data has allowed us to ensure that only the minimum number of animals will be used for 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.	Mice are the lowest vertebrate in which animal models have been established for these bacterial infections. A number of genetically modified animals deficient in pathways we intend to study are also available in mice and not higher animals. Extensive training will be provided to minimise welfare cost to animals and in depth guidelines for welfare have also been drawn up to aid refinement.

Project 5	**Molecular probes for plant cell walls**	
Key Words (max. 5 words)	Antibodies, cell walls, polysaccharides, pectin	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Plant cell walls are important cell components that underpin many aspects of plant and crop growth. Cell walls are constructed from an array of complex carbohydrates/polysaccharides. Knowledge is lacking on how these carbohydrates link together to form functional cell walls. Molecular probes such as antibodies are essential tools to study carbohydrates and determine how they function during plant growth.	
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)?	Understanding plant cell walls will increase knowledge of plant biology and the mechanisms that control how plants grow. This can be applied to crops to ensure sustainability and optimization of cropping systems. Cell wall carbohydrates are also the dietary fibres essential for human health. The molecular probes made in the project will also be applicable to the analysis of dietary fibre.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rat. It is expected to use 50 animals over the 5 years of the project.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The level of severity of the protocol is mild and no adverse effects are likely or anticipated. The animals will be killed at the end of the protocol.	

level of severity? What will happen to the animals at the end?	
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals, such as rats, provide the most effective antibodies to carbohydrates and plant fibres. Non-animal alternatives cannot produce molecular probes with the appropriate sensitivity and specificity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Only two animals will be used for each target carbohydrate. Immortal cell lines, secreting monoclonal antibodies will be isolated at the end of the procedure. These can produce antibodies indefinitely and no more animals will be required for a particular target carbohydrate.
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.	Rats are selected for monoclonal antibody generation as they provide an efficient system and have proven to be highly effective in the generation of specific antibodies to cell wall-derived molecules. Animal suffering is minimised by keeping animal use to a minimum and all procedures will be carried out by the same trained staff to minimize disruption.

Project 6	**Combating tapeworm parasitism using new model systems**	
Key Words (max. 5 words)	Tapeworms, parasitism, laboratory culture	
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)	Tapeworm parasitism of ourselves and of our domestic and farmed animals continues to be a global medical and economic burden. The development of vaccines/drugs to combat tapeworm infection relies on knowledge of the parasites genes and the pathways that the genes are involved in. We will use natural tapeworm parasites of rodents as a model system in the laboratory to characterise the genes that control the parasite's growth and reproduction, helping to reveal new targets for chemotherapeutic intervention.	
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 is ultimately aimed at identifying new targets for therapeutic intervention in order to reduce the global burden of tapeworm parasitism in ourselves and our domestic and farm animals. In addition, our work provides data that can be used immediately by other researchers in academia and industry for innumerable purposes. For example, by providing an 'encyclopaedia' of the genes and other features of the genome, our work saves enormous time, expense and use of animals that would otherwise be required for almost any contemporary research programme. For example, such data allow for 'virtual' (i.e. computer-based) screening of gene targets for existing drug compounds, and for the rapid	

	development of gene-specific probes. Our investigations also provide high-quality training in contemporary research skills for persons ranging from sixth-form students to post-graduate students and academic scholars.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five-year period we will use a maximum of 1,000 mice and 500 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Rodents will be infected with tapeworm larvae by oral gavage (i.e. orally administered by feeding tube; a procedure classed as mild severity) and killed 1-180 days post-infection. As natural hosts of these tapeworms for millions of years, rodents show no adverse symptom of infection.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to culture tapeworms specimens for research without the use of their natural hosts. While in vitro culture using synthetic nutrients can provide limited growth of worms, the full life cycle (involving both beetle and rodent hosts) cannot be maintained in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We only generate parasite material as needed, while maintaining a small number of infected mice (10) and rats (2) at all times to ensure continual passage of the parasite culture.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the only animals capable of hosting these parasites and are thus the most refined choice. Outside of infecting the rodents, we perform no procedure on the animals.

Project 7	**Mitochondrial biology of trypanosomes**	
Key Words (max. 5 words)	Trypanosomes, sleeping sickness, drug discovery	
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)	Public health and the economy in developing countries suffers from devastating infectious diseases of humans and their livestock - one of the reasons for the so-called “poverty trap”. Many of these diseases, such as sleeping sickness, affect mainly the poor and have largely been neglected for drug development by the pharmaceutical industry. Among the most important “neglected diseases” are those caused by trypanosomatid parasites. This Personal Project Licence (PPL) application covers two research projects, one of basic and one of translational or applied nature. The basic research project studies the mitochondrion in the sleeping sickness parasite, and the second aims to identify and optimise small molecule inhibitors of an essential parasite enzyme called RNA Editing Ligase 1 (REL1). These could later be developed into new drugs. Trypanosomatids are unicellular organisms that have a single mitochondrion - a cell organelle that is often described as the 'power plant' of eukaryotic cells. Mitochondria have their own genome (mtDNA), which is important for organelle function. A main goal of our research is to understand exactly what the proteins encoded in mtDNA actually do. This is complicated by the fact that trypanosomes have a complex life	

	cycle. They are transmitted by tsetse flies, and the trypanosome that thrives in the mammalian bloodstream (and makes the mammal sick) changes the purpose of its mitochondrion once it has been taken up by the fly. Investigating which of the mtDNA genes are needed by the parasite when it changes from the mammalian bloodstream form to the one that can survive in the midgut of the tsetse fly requires experimental infections in rodents. For the second project we need to test REL1 inhibitors that we are identifying in the lab in mice infected with trypanosomes. This enabled us to assess how well tolerated these compounds are in mammals and how effective they are in curing a trypanosome infection. REL1 is an essential enzyme in these parasites, so an effective REL1 inhibitor will kill the trypanosomes and cure the rodent.
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)?	A goal of this project is to help develop new drugs for diseases caused by trypanosomes. One promising approach in drug development is to identify a unique parasite enzyme and to then inhibit this enzyme with a drug. The enzyme's absence in humans will help avoid side effects. We previously identified such a unique enzyme, called "RNA editing ligase", and showed that survival of the sleeping sickness parasite depends on it. We will use computational as well as biochemical approaches to find strong inhibitors of this enzyme. Our hope is that such chemicals can then be developed into better drugs against diseases caused by trypanosomes. The basic research component of this project investigates the function of the parasite's mitochondrion, a cell organelle that is often described as the 'power plant' of eukaryotic cells (all non-bacterial organisms are called eukaryotes). Mitochondrial defects are linked to an increasing number of severe disorders in humans. Trypanosomes are very useful experimental models for improving our understanding how these important organelles work, not just in the parasite but in eukaryotic organisms in general. This research also has great potential to identify other unique parasite enzymes that in the future could be exploited as new drug targets, as described above.
What species and approximate numbers of animals do you expect to use	1440 mice and 240 rats over a 5-year period.

over what period of time?	
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?	Adverse effects associated with compound screening may include making the animal ill following administration of the compound. In extreme cases this may lead to the need to humanely kill the animal due to unacceptable side effects. All compounds will first be tested thoroughly in cell based assays and little toxicity is expected for compound that are selected for experiments using animals. All animals will be closely monitored and any animals showing signs of clinical distress will be euthanised. Adverse effects associated with infecting mice with trypanosomes include the animals carrying a high parasite burden and showing signs of being unwell as a result. Some of the parasite strains used in these experiments cause a progressive and highly predictable disease. Infected animals will be checked regularly by experienced staff with end points clearly defined to minimise suffering and disease progression beyond the specified severity limit. At the end of each experiment, all animals will be killed using humane methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Culture systems for trypanosomes exist and are in routine use in my laboratory. However, culture media require 10-30% fetal calf serum. Furthermore, parasite numbers are low compared to those that can be harvested from a single rodent infection and parasite forms produced by culturing are not biologically identical to the forms multiplying in the mammalian bloodstream. Thus, experimental data from cultured parasites are often of limited value. Extensive use of resources describing alternatives to experiments in animals, including PubMed, FRAME (the Fund for Replacement of Animals in Medical Experiments), and NC3R (National Centre for the Replacement, Refinement and Reduction of Animals in Research), failed to identify methods that can fully substitute experimental trypanosome infections in rodents. However, we are replacing rodent infections with parasite cultures whenever it is compatible with the scientific objectives of the study.
2. Reduction Explain how you will assure the use of minimum numbers	Whenever possible, we have applied statistical analyses to identify the minimal number of animals required to reach our scientific objectives. Also, we aim to reduce the number of animals required by

of animals	resorting to cultured parasites whenever it is compatible with the scientific objectives of the study. See *Replacement* for further details.
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 and rats are used for parasite infections. In almost all cases, mouse infections are used because how the infection develops and clinical signs of distress are highly predictable, methods to minimise animal suffering are well established, and staff at our local facility is well trained in managing these animals. This also allows comparison with experiments carried out in other laboratories, where mouse infections are the routine method for analysing parasite growth, virulence, drug efficacy etc. Where large numbers of parasites are needed (for example for biochemical studies) rat infections are used, since neither culturing methods not mouse infections can deliver the amount of biological material required for these studies.

Project 8	**Novel types of gene regulation in development**	
Key Words (max. 5 words)	Novel, types, gene, regulation, 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)	The regulation of genes is a key determinant of normal development of complex organisms and a failure in this process is often the molecular basis for disease. Some mechanisms of gene regulation are still poorly understood. The objective of this project is to understand two of these mechanisms more clearly by testing what effect removing individual components of these mechanisms from embryos has on their development.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main benefit is to basic knowledge: the more complete understanding of a very important biological process. The work will however reveal new DNA sequences and proteins that interact to control gene activity; these can be used as targets for small molecules with therapeutic potential in the longer term.	
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 300 adults and 4650 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 adverse effects and the likely/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 frogs can be used 15 times before they are finally	

level of severity? What will happen to the animals at the end?	killed humanely and their oocytes used for experiments. Up to 60 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	We are studying the effects of these poorly characterised forms of gene regulation on the whole organism and this requires embryos. These in turn need to be produced by adult animals. Whilst we could understand some of the effects using cell culture we would be unable to ascertain any effects on cell signalling and interactions between cells.
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. The numbers of tadpoles are based on the minimum numbers that we have been able to use for very similar experiments currently being performed.
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.	Xenopus are the species of choice for this work since their genome is now arguably the second best characterised one after humans and because proteins made in the developing oocyte can be removed much more simply than in other animals. To minimise the harm to animals we are using a new technique to re-introduce oocytes into female Xonopus; 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 9	**Developmental Biology and Evolution of cavefish**	
Key Words (max. 5 words)	tetra, development, evolution, adaptation	
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)	The research goal is to understand the generation of morphological novelties during evolution. The regulatory genes of morphological development have been investigated in many model organisms, such as fly, mouse, and zebrafish. Using these organisms, we have learned a great deal about the molecular mechanisms of morphogenesis. Although recent data suggest that the sequences of regulatory gene and genetic mechanisms are identical among vertebrates, how has morphological diversity evolved? Unfortunately, it is difficult to answer this question by simply comparing the data from the model organ i sins because of their phylogenetic distance from one another. The question of whether different mechanisms underlie morphological evolution within a species (microevolution) and above species (macroevolution) is controversial. One way to approach a solution is to first determine which developmental mechanisms are altered to allow morphological diversity within a species, and then to compare those developmental mechanisms among closely related species to find out whether the same developmental mechanisms have been targeted by macroevolution.	
What are the potential benefits likely to derive from this	Although the evolutionary mechanisms responsible for morphological changes have been discussed	

project (how science could be advanced or humans or animals could benefit from the project)?	since beIre 1)arwin, they are still a mystery. Our studies in this project will shed light on how genes function and regulation has been altered during the morphological evolution. Also, the investigation of neural degeneration in cavefish in this project will result in an increased understanding of the genes contributing to congenital neural defects in vertebrates, which includes humans. Studies in this project will increase our knowledge of how organisms have adapted to a new environment and may prove useful in understanding how organisms may deal with environmental changess resulting from global climate change.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use about 8000 cavefish and about 800 Danio rerio (zebrafish).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In this proposed project, micromanipulation will he performed for early embryos, and that may lead to some developmental adverse effects at the embryos. A wide range of early developmental changes could occur but most likely will involve mis-patterning of neural tissues. All embryos with significant adverse effects will be killed by schedule 1 methods. Therefore, significant adverse effects upon fry beyond the stage where the fry may feel pain are not expected. Expected level of severity of the animals is mild. At the end of the protocol, all animals will be killed by a Schedule I method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Most of our studies require analysis of the whole animal and these cannot be modelled in other ways. however, a great deal of work can be done on computers to help us to improve the design of our experiments. For instance, we are using zebrafish gene expression database for finding genes that express during neurogenesis. These genes are possible candidate for causing neuro-degeneration in the cavefish. This information helps us to select between various candidate genes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The cavefish have been newly developed as one model organisms for evolutionary research. We will provide embryos and tissue samples to other scientist in UK and other European countries. This will help to reduce the number of wild caught fish from their habitats. To reduce numbers of animal for

	experiments, we will employ several statistic methods in consultation with a mathematician in our department.
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.	Cavefish are one of the best model systems to investigate developmental mechanisms for adaptive evolution due to availability of embryos in a lab. Also, studying this species could help to understand many issues related with human health, such as eye and neurodegencrative disease, ability of heart regeneration, and obesity. Zebrafish is well researched for animal welfare. cavefish is very closely related with the zebrafish. We are tightly communicating with other zebrafish researchers to identify any new methods to improve animal welfare and to reduce suffering zebrafish. Adopting such a new technique for cavefish research will help us to improve the animal welfare and reducing suffering. Any fish with adverse phenotypes will be killed by schedule I. I will not be carrying out cell donations or transplantations once fish are at a stage that they can experience pain.

Project 10	**Rodent Models of Dementia: Cognition and Therapy**	
Key Words (max. 5 words)	Memory Dementia Therapy Downs Syndrome	
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)	Summary of key objectives Objective 1: Test the hypothesis that genetic mutations, associated with dementia and Down Syndrome (trisomy 21) related pathologies, will cause accelerated age-related changes in cognition and emotion. Objective 2: Test the impact of interventions either designed to tackle key pathological processes involved in dementia and intellectual dysfunction or enhance environmental factors (such as diet and exercise) that influence the brains susceptibility to cognitive dysfunction. Objective 3: Test the hypothesis that trisomy of different regions of chromosome 21 contribute differentially to the development of the range of cognitive/emotional impairments and dementia found in Down syndrome. Objective 4: Develop novel tests of cognitive and emotional function in animals that map onto cognitive processes in disrupted in patients. <u>Rationale</u> Dementia affects currently over 850,000 people in the UK and there is no treatment for this	

	disease. The cost of dementia care to the UK economy is estimated to be £26.3 billion year. The Kings Fund estimate that this may increase to £34.8 billion by 2026. The main aims of this project are to examine the key pathological processes (both genetic and environmental) that are hypothesised to cause or increase the risk of dementia. The project will examine the impact of environmental factors, such as exercise, on brain mechanisms supporting memory in mouse models of dementia. In addition, we will examine the efficacy of a novel antibody therapy that targets a specific pathway in the generation of amyloid (a key pathological event) on the development of brain abnormalities and cognitive decline in mice expressing human mutations linked to dementia. One of the key genetic causes of early-onset dementia was discovered in individuals with Down Syndrome (DS). Indeed, individuals with DS may develop dementia as early as 40 years of age and is associated with overexpression of the gene that encodes for the amyloid precursor protein (APP) on chromosome 21. Research into the genetic causes of DS continues to provide important information about the contribution of other chromosome 21 genes to dementia and intellectual dysfunction. However, the main causes of intellectual impairment in DS remains unknown and one of the aims of this program is to evaluate the different contributions of sub-regions of genes on chromosome 21 to the range of cognitive changes associated with this syndrome. To achieve this objective we will develop appropriate novel cognitive test for animals and will characterise cognitive and emotional function in mouse lines that have selective trisomy of different sub-regions of the genes on chromosome 21. We will also test hypotheses concerning the likely neural mechanisms that cause intellectual dysfunction and the impact of putative interventions, such as exercise and modulation of receptor activity.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This research will provide a better understanding of how dementia affects cognition from the level of individual neurons to the systems level. The use of MRI technology will allow use to generalise our work in characterising the role of genes and evaluating therapies more easily to similar studies in humans. We will continue to examine a novel (patented) antibody therapy that reverses cognitive deficits in mouse models of amyloid pathology (a mouse model

	early stage dementia) and evaluate its impact on cognition in dementia in Down Syndrome animal models. This novel therapy may provide a future drug development pipeline for industry and the aim is to eventually licence the compound to Pharma. We will also establish the nature of the genes responsible for intellectual dysfunction in trisomy 21 and evaluate potential therapies (environmental and drug related) that combat these abnormalities.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats 1100 Mice 1500 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 goal of this project is to understand how dementia-related genes influence the neural process underpinning learning and memory and evaluate potential therapies that target these processes. Given that the main readout from our experiments reflect subtle cognitive changes in learning and memory and will not influence maintenance behaviours, such as feeding and grooming, the procedure we employ are frequently mild or moderate and very often may cause only acute discomfort. The project will either breed or obtain rodent models of dementia and Down syndrome (mild). These animals will then undergo behavioural testing on tasks that are designed to interrogate specific aspects of cognition and brain systems that are affected by the disorders. On a small number of occasions during the project, animals may be tested on a procedure in which they are presented with inescapable foot shock (severe). The conditions of shock delivery will often be very brief and will not be at a level to cause physical harm. Other procedures may involve the use of food or water restriction to motivate the animals to press a lever for food, for example when investigating reversal learning, or learning that a signal reliably precedes the delivery of a reward. The likely adverse effects of these manipulations are minimal (<1%). Some animals may receive a treatment designed to combat the effects of genetic modification, for example an antibody therapy. This may involve the use of surgical procedures, e.g., to allow drugs to be targeted to specific brain regions. The potential adverse effects of these manipulations may include changes in body

	weight and post-operative infections, but their incidence is very low (<1%). None of the procedures are designed to target the special senses or influence maintenance behaviours, such as eating and drinking. Once they have finished behavioural testing, rodents may undergo magnetic resonance imagining in order to foster the translation of our cognitive and brain function changes to assays of human brain function. At the end of each experiment, brain tissue will be collected and the samples analysed for pathological markers, such as amyloid production or other proteins linked to abnormal neuronal function, in order to characterise the mechanism(s) of action of both genes and putative treatments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project aims to understand how genes linked to dementia and intellectual dysfunction in humans leads to cognitive impairment. To fully understand the gene-brain-behaviour relationship requires the use of live animals. Furthermore, the success of any treatment for these conditions in humans relies upon not cognitive changes in the patient. Therefore work with live animals provides a direct means to link gene function, treatment to a cognitive read out relevant to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	i) By the use of within-subject comparisons to improve statistical power. ii) By using well characterised behavioural tasks, which provides reliable measures of cognition and relating these closely with procedures carried with humans. iii) By combining techniques in the same animal, e.g., the use of non-invasive imaging techniques (e.g., MRI) that permit repeat analyses with behavioural techniques designed to engage specific cognitive processes. iv) Using animal models based on current understanding and genetic technology.
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	Rodents are used because they allow the application of the most sensitive and well-understood techniques for assessing cognitive processes in combination with an analysis of neural systems that underpin them and are also suitable for investigating aging-related brain changes. Rodents have been the dominant species with which to undertake genetic

measures you will take to minimise welfare costs (harms) to the animals.	modification. While their popularity has increased with the advance of genetic technologies the analysis of mouse behavioural phenotypes, in particular, has lagged behind to some extent. One aim of the project is to address this within the context of modelling cognitive function sensitive to dementia and Down Syndrome. Genetically modified mice expressing human genes linked to Alzheimer's disease (such as APP mutations) have been used extensively – especially in terms of modelling very early changes in brain function associated with dementia. This is arguably an appropriate point to test therapies because of their potential for long-term benefit to humans. Our work will continue in this regard to examine the impact of environmental and pathology pathway specific interventions to modify the onset and progression of cognitive change. Likewise, genetically modified mice provide a refined tool to dissect the contribution of triplication within specific regions of chromosome 21 to impaired intellectual function. Harm to the animals will be minimised by selecting the least invasive means for achieving our experimental goals. The use of procedures rated as "severe" will be kept to an absolute minimum. Indeed the majority of behavioural work will use relatively mild procedures, such a food restriction. Surgical procedures will be carried out to manipulate specific brain pathways or synaptic processes thought to underpin cognition. These techniques will be used to establish the necessary brain systems contributing to cognition. The animals will receive appropriate anaesthetic, post-operative pain relief and close monitoring to ensure full recovery and no interference with vital maintenance behaviours. Once again extensive literature searches and laboratory experience will be used to ensure that unexpected adverse effects of a manipulation are minimal. We have extensive experience working with mice and thus are able to determine the minimum number of animals required to achieve sufficient statistical power to evaluate our hypotheses. The animals will be constantly monitored using animals house standard operating procedures, such as health checks, to ensure their well-being. Any events that lead to unexpected harm to the animal will be stopped.