Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 20

Projects with a primary purpose of: Basic Research – Respiratory System

Project Titles and keywords

1. **Mucosal Immunity**
    - Immunity, Infection, respiratory, mucosal, disease

2. **Antivirals directed against respiratory viruses**
    - Antiviral, respiratory, virus, treatment

3. **Investigations into poxviruses**
    - Poxvirus, Vaccinia virus

4. **Pathogenesis of Respiratory and Persistent Virus Infections**
    - Virus, Respiratory, Vaccination, Anti-virals.

5. **Functional Electrical Stimulation of respiratory muscles in horses**
    - Horse, functional electrical stimulation, respiratory

Project 1	**Mucosal Immunity**	
Key Words (max. 5 words)	Immunity, Infection, respiratory, mucosal, disease.	
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)	We will examine the importance of genetic differences, which occur naturally in humans and relates to immunity and susceptibility to infections and diseases. Using genetically altered mice, which mimic these genetic differences, we can look for treatments which exploit naturally occurring and recombinant immune defence molecules.	
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 few treatments for lung disease, such as; chronic obstructive pulmonary disease (COPD), neonatal chronic lung disease and emphysema. Many treatments are ineffective because the disease is so complex and differs from patient to patient. Altered levels of immune molecules have been associated with many lung infections and disease. By using genetically altered mice we can investigate immune mechanisms which contribute to disease We have shown that inflammatory responses can be targeted to specific infections and disease and also that innate immune molecules can help to prevent initial infection and help with the healing process afterwards.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice were chosen as they are the lowest vertebrate group on which well characterized lung disease investigations have previously been carried out. We aim to use no more than 1000 mice per year for 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?	Our studies involve the exploitation of innate immune molecules that are expressed in the body. We have discovered the benefits of re-introducing these proteins where there is little or no protein expression and as such we expect few adverse effects. Cold stress and wellbeing is assessed by using a scoring sheet for the monitoring the mice under procedure and appropriate action will be taken, such as terminating the experiment, if there is evidence of any distress. Anaphylaxis is a potential adverse event which will be controlled by close monitoring of the animals undergoing procedure. Saliva sampling should cause no ill-effects, and ear notching involves slight, transient pain, without healing problems. Blood sampling will not exceed 15% Total Blood Volume in any 28 day period. When work under terminal anesthesia is involved, the level of anesthesia will be maintained at sufficient depth for the animal to feel no pain. Animals will be terminated at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We carry out experiments using these reagents, which have proved successful using *in vitro* (non-animal) systems. We have shown that inflammatory responses can be manipulated to target specific infections and alleviated disease. We see that innate immune molecules can help to prevent initial infection and help with the healing process afterwards. We now need to explore if we can mimic these effects within the complexity of the whole lung.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We produce experimental plans with the help of animal specialists and statisticians to ensure we do not use more animals than needed to see any effects. We carry out pilot investigations to determine the minimum dose that shows an effect, doses are based on previous published *in vivo* results and data to minimize numbers and suffering.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice were chosen as they are the lowest vertebrate group on which well characterized lung disease investigations have previously been carried out. We carry out pilot studies and refine protocols to reduce numbers, pain and suffering. When setting up new investigations, we use stress monitoring charts with advice from animal experts. We update our protocols based on current knowledge ensure the least evasive methods of experimentation

Project 2	**Antivirals directed against respiratory viruses**	
Key Words (max. 5 words)	Antiviral, respiratory, virus, treatment	
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)	Respiratory virus infections are a major healthcare burden across the world. While these frequently cause a mild illness that ultimately resolves, the most severe disease occurs predominantly in the very young and the elderly where infection can be fatal. In more severe cases in patients of any age the infection is typically referred to as influenza-like illness, though it may or may not be the result of an influenza virus infection. Human respiratory syncytial virus (HRSV) is the predominant aetiological agent of respiratory infection resulting in hospitalisation of infants worldwide. HRSV infections occur in annual winter epidemics in temperate climates with an estimated 64 million cases and 160,000 deaths worldwide every year. Influenza virus infections also occur in annual winter epidemics with an estimated three to five million cases of severe illness and 250,000 to 500,000 deaths annually. In addition influenza A viruses cause worldwide pandemics that result in a greatly increased levels of morbidity and mortality over the initial 1-2 years of the new 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)?	Other viruses associated with acute respiratory disease include the parainfluenza viruses, adenoviruses and coronaviruses. Respiratory virus infections in domestic livestock also represent a significant economic and disease burden with estimates of up to 5.5% mortality in cattle. Bovine respiratory syncytial virus (BRSV) is a major problem generating losses of more than $55 million to UK industry. The paucity of antiviral therapie for treatment	

	of acute respiratory virus infections and the increasing appearance of drug resistance have generated an urgent clinical and agricultural need for new anti-virals. The aim of the project is to identify candidate treatments to address this need. This will be achieved by characterising the antiviral effects of specific compounds and materials on respiratory virus disease in mice prior to clinical and large animal trials. Materials will be tested for their ability to reduce virus replication and/or reduce observable signs of respiratory virus disease in a number of virus systems including, but not restricted to, HRSV, BRSV, PVM, influenza viruses and paramyxoviruses. The compounds and materials will be targeted against specific virus- encoded functions or host cell functions essential for virus replication.
What species and approximate numbers of animals do you expect to use over what period of time?	All experiments on respiratory virus infection will be conducted using mice. It is anticipated that a maximum of 13,500 mice will be used during the lifetime of the project. In addition influenza virus stocks will be prepared using embryonated chicken's eggs with a maximum of 3000 eggs at greater than 2/3 the full gestation period anticipated to be used during 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?	All animals will be checked visually following recovery from anaesthesia and daily thereafter. No adverse effects of anaesthesia are anticipated. As the work will investigate approaches to treat respiratory virus disease it requires the production of adverse responses in mice. Following infection with a respiratory virus all animals will be checked daily for the presence of symptoms of disease. It is expected that only those infected animals not receiving treatment with antiviral compounds will present with significant symptoms of disease. The progress of respiratory virus infection in mice is well understood and can be monitored by external signs which include poor coat condition, reduced activity and increased rates of breathing. A combination of these external signs of respiratory disease reflects the potential outcome of the infection and can be used to identify, in advance, any animals unlikely to survive the infection. With some strains of mice it is possible to monitor weight loss in conjunction with the external signs of disease and this additional measurement will also be carried out. Any animals observed displaying signs of distress or becoming very sick in the days following virus infection will be killed immediately

	using a schedule 1 method to prevent further suffering. A daily measurement of the weight of the groups of animals will be recorded and if any groups of mice show a weight loss exceeding 25% of the maximum weight they will be immediately killed by a schedule 1 method. While every effort will be made to cull mice in the late stages of disease it is not possible to completely eliminate death. For these reasons the severity is assessed as severe. Based on experience and refinement of a clinical score system used to assess the severity of infection over the last 5 years, fewer than 10% of the animals in the studies will die as a direct result of the virus infection. At the end of each experiment all animals will be killed using a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project seeks to investigate the protection from respiratory virus disease. Assessment of the onset and progression of disease is only possible in whole animals which can display the full extent of the pathogenic process. No in vitro surrogates for protection have been identified.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals necessary to provide statistically robust data will be used. This will eliminate the need for repetition of experiments, each of which would require the use of control animals. Experiments will be carried out concurrently wherever possible to permit the use of a single control group for several experiments and thus reduce experimental variation. Power calculations have been used to identify the minimum number of animals per group required to provide statistically robust datasets. These will be used to inform experimental design.
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 most accessible small animal model system for studies of respiratory virus disease. A considerable body of literature has been generated using mouse infection with respiratory viruses and this will provide substantial dataset with which to compare the results of the project. All experimental animals including uninfected controls will be monitored daily for signs of infection and the severity of the disease scored using a standard protocol. Mice showing the most severe symptoms will be culled using a schedule 1 method. Additionally,

	all groups of animals will be weighed daily and if the group weight falls below 25% of the maximum weight they will be culled.

Project 3	**Investigations into poxviruses**	
Key Words (max. 5 words)	Poxvirus, Vaccinia virus	
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)	The project aims to characterise the interactions between poxviruses and the infected cell, particularly at the molecular level. It will contribute to a more complete understanding of interactions between a virus and its host.	
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 knowledge gained from this project will contribute to the development of poxviruses as vaccine vectors and as agents to kill cancer cells. We will also be able to extrapolate our findings to other similar viruses such as African Swine Fever virus.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 200	
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 intradermal model will cause localised adverse effects of mild severity. A small area of inflammation will occur on the ear pinna of the mouse. The intranasal model will cause systemic disease of moderate severity characterised by weight loss, hunched posture and ruffled fur. Animals will be euthanased at the end of the	

	experiment using a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to fully characterise the molecular pathology of poxvirus disease in the absence of a complete host anti-viral immune response, particularly since some of the cellular and viral proteins we will be investigating will be immunomodulatory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be based on the minimum number required to achieve statistical significance with adequate power. This will be calculated for each study.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Murine models of poxviral disease are well described with extensive downstream investigation protocols available. Harm to animals will be minimised by regular monitoring, clinical scoring, and optimising the infective dose used.

Project 4	**Pathogenesis of Respiratory and Persistent Virus Infections**	
Key Words (max. 5 words)	Virus. Respiratory. Vaccination. Anti-virals.	
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)	This programme of work aims to study the how the body responds to virus infections and use this information to investigate the potential new vaccinations and drug treatments. The viruses that we are studying are Influenza A virus (lAy), human respiratory syncytial virus (HRSV) and gammaherpesviruses (yHV).	
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 viruses we are studying cause significant disease and death worldwide. Influenza causes seasonal outbreaks of disease and deaths in the elderly with occasional worldwide outbreaks that cause thousands of deaths each year. HRSV causes seasonal colds but is a major problem in newly born children, causes lung inflammation and can lead to death. Gammaherpesviruses cause cancers. There are still large gaps in our knowledge of how these viruses cause disease and even where available, vaccines and antiviral drugs are not 100% effective due to virus evolution. This project will advance this knowledge and help us to design and test new drugs and vaccines. This will lead to reduced disease and deaths due to virus disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of our experiments will be conducted in mice because of the availability of genetically altered strains, however, a small number of rabbits, wood mice and guinea pigs will also be used. We anticipate that 2450 mice, 250 wood mice, 30 rabbits and 100	

	guinea pigs will be used in experimental procedures 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?	Rabbits will be inoculated with protein samples combined with a adjuvant compound in order to produce antibodies that recognise the proteins. In rare cases, an abscess may form at the point of inoculation and this will be checked regularly. Mice, wood mice and guinea pigs will be infected with viruses and these will cause disease in the lungs and sometimes cells of the immune system. They will usually be maintained over a period of 2 weeks. The general condition of animals and their weight will be regularly monitored. Many of the animals will lose weight and then recover. About half of the mice will show no apparent sign of infection whereas the other half may show a mild flu-like illness. Some mice infected with gammaherpesviruses may develop tumours. This can occur in up to 20% of mice I year after infection. Tumours present as swollen stomachs. Mice will be monitored specifically for tumour development. In a small number of mice we will alter the immune response. In these mice a more profound or rapid disease could develop after virus infection. In this case, the general condition of animals and their weight will be regularly checked. About half of the mice will show no apparent sign of infection whereas the other half may show a mild flu-like illness. All animals at the end of every procedure will be humanely killed and tissues will be taken for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst we use cells cultured in the laboratory to study viruses and these experiments are informative, they are not currently able to mirror the complex environment found in the lungs. The interactions of multiple types of cells that form the lining of the lung, with cells of the immune system are needed to for our studies. These interactions are currently not possible in cell culture experiments in the laboratory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, we will use cultured cells and human tissues to address our research questions. When animal experiments are necessary, the number of animals necessary will be determined by statistical

	analysis, consulting with a professional statistician. This will ensure that we use the minimum number of animals necessary for our experiments to be statistically significant.
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 structure and function of the lungs of mice and guinea pigs is similar to that of the human. There are also many genetically altered strains of mice available that are suitable for the proposed studies. Therefore, the majority of our studies will be conducted in mice. Data from our previous and future experiments will be used to determine the minimum doses of substances required to work but avoid side effects. The shortest possible time of treatment will also be used to obtain experimental data Other species such as zebra fish and drosophila (flies) are increasingly being used instead of mice. However, neither of these alternative models have a respiratory tract or lungs and thus are unsuitable for our studies.

Project 5	**Functional Electrical Stimulation of respiratory muscles in horses**	
Key Words (max. 5 words)	Horse, functional electrical stimulation, respiratory	
Expected duration of the project (yrs)	5 yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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 aim to develop a totally implantable, functional electrical stimulation (FES) system for the treatment of naturally occurring equine upper airway diseases that will improve outcomes and welfare of horses with these 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)?	Development of these FES systems will allow more efficacious treatment of equine upper respiratory disease, reduce wastage of horses with these diseases and improve the welfare of horses affected with these common diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 16 adult horses 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 implantation of electrodes and totally implanted FES devises is performed under general anaesthesia, mild incisional effects can occur but are transient and pain is minimised by the use of anti-inflammatory medication. Intermittently, percutaneous needles are transiently inserted into muscles to record EMG and inject local anaesthetic, which does not cause adverse effects. Horses will be humanely euthanized at the end of the study.	

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 because, whilst the diseases are similar to some human diseases, we need to test the FES systems in naturally occurring equine disease before they can be used in clinical patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of horses by using non-invasive ways of longitudinally measuring changes in muscles to functional electrical stimulation, rather than biopsies or post-mortem evaluation.
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 are using horses with naturally occurring disease so that no models of disease are required, and will allow us to move straight from limited testing to treating client owned horses in clinical trials. We will minimise welfare costs by using totally implantable systems and allow horses regular access to grazing during the study period.