Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 5

Projects with a primary purpose of: Translational and applied research – Human respiratory disorders

Project Titles and keywords

1. **Gene Therapy For Cystic Fibrosis & Other Diseases**
    - Gene Therapy, Vectors, Lung

2. **Toxicology and MedCM for Inhaled Chemicals**
    - Chemical, medical countermeasures, pig, lung

3. **Modifying Respiration in Rats using Stimulation**
    - Asthma, spinal cord stimulation

4. **Respiratory Diseases**
    - Respiratory, Inflammation, In-vivo, Infection, PK/PD

5. **Development of new therapies for diseases affecting the lung**
    - Inhalation, gene-based therapies, lung

6. **Animal Models of Human Disease**
    - Animal models, new drugs, efficacy, safety

7. **Respiratory Pharmacology**
    - Respiratory diseases, lung, inflammation, rodent

8. **Airway biology**
    - Asthma, lung inflammation, therapeutics

<table>
<tr><td>Project 1</td><td colspan="3">Gene Therapy For Cystic Fibrosis & Other Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Gene Therapy, Vectors, Lung</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>~~No~~</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Gene therapy, in its simplest form, is the transfer of DNA encoding a functional gene into human cells that contain a mutated gene in order to treat a genetic disease. Successful human gene therapy has been achieved in a range of rare diseases associated with blindness and blood disorders. The objective of the project is to investigate the use of gene therapy to treat a range of lung and other human diseases.

Our most advanced project is the development of a gene therapy for the inherited disease cystic fibrosis (CF). CF affects ~80,000 people worldwide with ~9,000 of these in the UK. Under previous Home Office project licences, we have developed a gene therapy that is now being tested in late-stage clinical trials in CF patients. We are developing new gene therapy formulations that we hope will be</td></tr>
</table>

	more effective and longer lasting (perhaps yearly rather than monthly doses). We are now also intending to develop gene therapy for other inherited diseases of the lung such as emphysema, for lung infections such as influenza and for diseases outside of the lung such as haemophilia.
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 targeted human diseases are associated with a high burden of care (daily physiotherapy sessions for certain lung diseases, frequent injections for other diseases) and considerable costs to the NHS (ranging between £15K and £500K per patient). A potent gene therapy for any of these diseases will provide a significant improvement in the quality of life of affected individuals and a reduction in NHS costs.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year project we expect to use a maximum of 15,000 mice and 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?	Under an existing Home Office project licence, we already perform similar studies and thus we have a good idea of the expected adverse events, which are typically mild, transient “flu-like” symptoms that resolve spontaneously within days. Overall, the severity limit is moderate. However, for >99% of the animals we expect that a mild severity limit would be appropriate. At the end of the studies, all animals will be killed under terminal anaesthesia or by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we use cell culture experiments to evaluate our gene therapy formulations. However, no cell culture models currently available recreates all aspects of the interaction between the lung and other organs we are trying to treat.
2. Reduction Explain how you will assure	We use statistical methods to minimise the number animals used in each and every experiment.

the use of minimum numbers of animals	Following advances in low-light technology, we are now able to measure the success of gene therapy experiments using non-invasive, highly sensitive cameras detecting bioluminescence light generated by successful gene delivery Consequently, we are now able to make several measurements over time in each animal. This has greatly reduced the number of animals used in our 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.	We will use mice and rats for these studies. We and others agree that rodents provide good models for the human diseases we intend to study. We have found that the observations made in previous studies with rodents have been good indicators of what occurs when we deliver our gene therapy formulations to humans. We expect the results generated in this project will be used to inform further clinical trials, and ultimately lead to new medicines to treat disease. We anticipate that some animals may experience a moderate amount of pain and/or discomfort. We will use appropriate analgesia to minimise these effects. Normal laboratory rodents will be used for the majority of the experiments. However, genetically altered mice will also be used. For the majority of these animals, the genetic alteration will have little or no impact on their wellbeing. However certain CF mice have significant intestinal problems, which dramatically shortens their life expectancy. We have in the past used such animals, but will in this project only use CF mice in which this intestinal problem has been corrected with a second genetic alteration. Such “intestinally corrected” CF animals have a normal life expectancy and no other untoward health issues. The experimental procedures allow delivery of the gene therapy reagents via a wide range of methods, However we will where possible use an aerosol delivery method in which the reagents are delivered as a mist to be breathed in. Our experience with this approach suggests the animals

	treated in this way suffer no distress and it mimics the delivery system used clinically. All animals will be monitored on a regular basis and any animal showing signs of distress will be promptly killed.

Project 2	**Toxicology and MedCM for Inhaled Chemicals**	
Key Words (max. 5 words)	Chemical, medical countermeasures, pig, lung	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	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)	Exposure to toxic chemicals can occur as a result of accidents within the home, industrially, on our road and rail networks, or as a result of terrorist or insurgent activity. As military operations in urban and industrialised environments increase, service personnel are also at risk. On breathing in the toxic chemical, the lungs can become so badly damaged that they can no longer deliver oxygen to the body, and death can result from this lack of oxygen. The mechanism by which each toxic chemical damages the lungs is not fully understood. Once lung injury has occurred there are no specific drug treatments and in severe cases the patient would need to be hospitalised in an intensive care unit. As there are only limited numbers of ventilators and specialist nursing the NHS and military hospitals could very quickly become overwhelmed should there be large numbers of casualties. The aim of the work covered by this Licence is to assess the lung injury produced by inhalation of the toxic chemicals in order to better understand the way in which the injury occurs and test potential treatments. This will provide information on biological	

	pathways that are affected and could be the target for drugs or therapeutic interventions.
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)?	Identification of treatments for acute lung injury will reduce or eliminate the requirement for resource intensive respiratory support within an intensive care environment. This will improve the chances of survival and subsequent quality of life of those with lung injury caused by inhalation of chemicals.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 450 pigs over the 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?	Under Protocol 1 animals will be terminally anaesthetised (i.e. anaesthetised for the entirety of the study) and as such should not experience any adverse effects or pain from the surgical procedures or administration of the toxic chemicals. On completion of the study the animals will be killed without recovering consciousness. Under Protocol 2, animals will be anaesthetised for surgery to put catheters into blood vessels to allow blood sampling. They may also have a telemetry device implanted. The animals will then be recovered from anaesthesia. Both these procedures enable the conscious animal to be monitored while performing normal behaviours e.g. eating, drinking, and moving freely within their home environment. This will cause some transient discomfort but pain killers and antibiotics will be administered. Following a suitable period of recovery the animals will again be anaesthetised in order to safely expose them to a toxic chemical. On recovery from exposure these animals are likely to experience signs of poisoning associated with chemical exposure, the major sign being difficulty in breathing due to lung damage. Animals which experience difficulty in breathing typically demonstrate increased respiratory rate and effort of breathing. The severity of these signs will depend upon the amount of chemical administered and the effectiveness of the treatment. Animals will be killed at either a humane endpoint or 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	Unlike other areas of pharmaceutical development, few opportunities exist to demonstrate the effectiveness of drugs against toxic chemicals in humans. This makes the extrapolation of animal-derived data to man much more important. The physiological effects of chemical poisoning are complex, involving a number of organs and systems, and cannot be replicated fully *in vitro* (e.g. cell culture). Hence, such experiments need to be conducted in whole animals to study the interactions of these systems. Small animals can be used to assess mechanisms of injury and screen candidate therapies, however extrapolation of therapeutic benefit to man requires verification in a second species, which more closely represents exposure in man. By using pigs it is possible to assess therapeutic efficacy using human intensive care equipment which provides a more detailed picture of the effectiveness, or otherwise, of the drugs being tested, and greater confidence in extrapolation of benefit to man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using a large animal will reduce the overall number of animals used as multiple parameters will be monitored in each individual animal maximising the amount of data that can be obtained. It is not possible to measure all these parameters in a single small animal. In addition the ability to use human intensive care equipment and investigate human intensive care medical management strategies is of great benefit. This provides greater confidence in extrapolating results from these studies to man. Experience and power calculations using data obtained from previous studies will ensure that the minimum number of animals is used to obtain statistically significant changes in clinically important measures e.g. oxygen content within the blood, compared to air or chemical exposed control animals. Statistical power calculations will be made for any

	new chemical studied. Where possible, therapeutic drugs which have previously shown efficacy in small animal studies will be evaluated. Proof of principle studies will, if appropriate, be performed in the terminally anaesthetised animal prior to transitioning into the conscious animal.
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 have chosen pigs because we know that the physiology and physiological defence mechanisms are similar to those in man. The anatomy of the pig lung is also more similar to man; by by-passing the upper respiratory tract exposures to chemicals directly into the lung reflect human exposures more closely. We have extensive knowledge and expertise in the use of the pig in studies assessing mechanisms and drug treatments for chemically-induced lung injury. Use of human intensive care equipment within an intensive care-like setting allows in depth pathophysiological assessment of lung injury with more relevant extrapolation of clinical strategies to man. Animals will be assessed by experienced personnel who will ensure that, in the terminally anaesthetised pig, the level of anaesthesia is maintained throughout the 24 hour study to ensure the animal is unaware of the procedures being performed on it. In conscious animal studies experienced personnel will be familiar with the animal's normal behaviours and will assess the development of lung injury or therapeutic efficacy using a system which grades a number of clinical variables e.g. amount of oxygen in the blood, or difficulty in breathing, from normal baseline levels. Our previous experience using conscious animals has identified immediate cull criteria if signs associated with unacceptable suffering are present. Continuous open mouth breathing and distressed vocalisation are considered to indicate unacceptable suffering. Animals will be killed immediately (Schedule 1) if they demonstrate these signs.

<table>
<tr><td>Project 3</td><td colspan="3">Modifying Respiration in Rats using Stimulation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Asthma, spinal cord stimulation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Asthma is a common condition affecting approximately 10% of adults and up to 30% of children. Acute, severe asthma remains a significant cause of preventable death, with the majority of deaths occurring before hospital admission. An asthma attack may be acute (over a period of minutes) or may have an indolent course over days, but one of the most striking features of a serious attack is that there is often a dissociation between the perception of the severity of the attack and the actual danger . This may lead to an asthmatic patient failing to seek help early enough to prevent death. Approximately 1200 people per year in the UK die from an acute asthma attack (http://www.rcplondon.ac.uk/projects/national-review-asthma-deaths) although this may be an underestimate of the real figure. The aim of this project is to test the feasibility of electrically</td></tr>
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	stimulating the nervous system in order to reduce the bronchospasm associated with asthma. Proof of principle exists from work performed in the 1970s but before the advent of modern technology. The clinical need is the large number of asthmatic patients (and potentially those with other lung diseases such as chronic obstructive pulmonary disease) who have 'brittle' disease (poorly controlled with a high risk of death) with a high rate of admission to Intensive Care or indeed death. The aim is to develop an implantable system that can both measure respiratory distress and respond to it by opening the airways via nervous stimulation. An implantable system that responds to abnormal physiology is an attractive therapy as it would not rely on the patient making a decision or being able to respond to the need for immediate action. In addition, such a system could have built in telemetry to inform the clinician remotely that there is a problem.
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 are that patients may feel symptomatically better from the treatment and have a reduced hospital admission rate and death rate.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 110 rats 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 level of severity? What will happen to the animals at the end?	About two-thirds of the animals are likely to experience adverse effects that are of moderate severity. They will be implanted with a stimulating electrode into the spine (overlying the spinal cord) which will be stimulated to see if the induced 'bronchospasm' (wheeziness) is reduced by stimulation. Adverse effects include post-surgical pain including wound pain. This will be treated with analgesia pre- and post-operatively (routinely). The breathing experiments involve putting the rats in a glass chamber whilst awake and manipulating the

	breathed gases so that they inhale Methacholine. Methacholine is a drug that induces bronchospasm and therefore causes wheeziness. Some of the rats will be made 'asthmatic' by exposing them to allergens (things that irritate the immune system). This will make the animals become wheezy for a short time when exposed to methacholine but otherwise they will be comfortable.The aim is for the stimulation to reduce the breathlessness. At the end of the experiments, they will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because we are testing the effects of a device on a disease state, animals are necessary to provide a realistic physiological (and pathological) system. Human experiments are not possible at this stage because of the unknown effects of such a prototype. Work on the stimulator system itself will be done prior to the start of the project; we will seek to make further refinements in the light of the experimental data as the work progresses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project has been designed such that each stage depends on success in the previous one. For example, testing with Methacholine will only occur once the implantable system has been refined for use in this situation. Rats will act as their own controls because we are able to turn the stimulator on or off. Therefore, there is no need for a control group in the awake experiments but some non-asthmatic rats will be used for the terminal experiments in order to reduce the exposure of rats to wheeziness where it is not necessary.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat is the lowest and least sentient animal that can be used for these procedures. Smaller animals would be too small to produce a stimulator system for the spinal cord. The rat is also large enough to be translatable to humans. Welfare costs will be minimised by subjecting each animal to as few steps as possible. The experiments will be carried out within 8 weeks of the initial asthma model induction so as not to prolong follow up observations. Other refinements include the routine

	use of pre- and post-operative analgesia, good peri-operative care including heat pads and fluid administration, adequate housing with environmental enrichment, and the integration of veterinary advice and services to improve the welfare of the animals.

<table>
<tr><td>Project 4</td><td>Respiratory Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Respiratory, Inflammation, In-vivo, Infection, PK/PD</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</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 this licence is to discover new molecules that can be developed into medicines for the treatment of respiratory diseases such as asthma, and chronic obstructive pulmonary disease.

This will be done by:

1. Learning more about the physiological alterations that result in respiratory disease

2. Identifying molecules that will interfere in the pathways thought to cause the disease

3. Developing new ways to achieve 1&2 using the smallest number of animals and the least invasive procedures</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>The potential benefits of this project are that by producing high quality data this will provide key support in the development of new, better</td></tr>
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advanced or humans or animals could benefit from the project)?	medicines to treat people with respiratory diseases such as asthma, and chronic obstructive pulmonary disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect that up to 21,000 mice and 11,000 rats 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?	Typical studies can last from 1 day up to 7 weeks. Animals will be dosed with inflammatory substances and/or micro-organisms to produce changes in the lungs. We will measure the effect of pharmaceutical substances on the resulting airway inflammation, change in lung function and levels of substances within the blood and tissues. These studies will allow us to understand more about both the disease mechanisms we are investigating and to identify and optimise new substances to interact with that mechanism and lead to a new drug for patients with that disease. The majority of animals will experience no adverse effects. Up to 10% of animals may experience moderate severity e.g. laboured breathing, in response to inflammatory challenge, micro-organism infection or substance effects. Studies will be designed so the minimum number of animals experience pain and distress. Animals will be observed regularly to monitor changes in appearance and behaviour and appropriate action will be taken to alleviate any pain and distress, e.g. administration of analgesia, withdrawal of the animal from study, or euthanasia if symptoms cannot be alleviated. All surgical procedures will follow the LASA principles for aseptic techniques. If any post surgical complications e.g. wound breakdown/repair, infection, cannot be remedied promptly and successfully using no more than minor interventions then the effected animal will be humanely killed. At the end of each study animals 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	This data cannot be generated without using animals because of the many and complex interactions that occur between inflammatory responses and the function of the airways. While a wide range of *in vitro* and *ex vivo* data is used to increase our understanding of a target in an isolated organ or tissue, understanding the integrated response in a whole animal is vital to guide progression to human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice will be sought to ensure the appropriate number of animals are used to ensure maximum value can be gained from pharmacodynamic data on a substance i.e. what effect it has on the body. Corresponding pharmacokinetic data, i.e. what effect the body has on a substance, will also be measured ideally within the same study or alternatively from a parallel series of studies. Additionally imaging may be used in some study designs to further reduce animal numbers by measuring in-life lung inflammation in the same animals at key points throughout the study. However imaging does require brief periods of general anaesthesia and for this reason is not used for all studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The animals and types of studies used for this programme of work have been chosen and developed to represent the least severe way of measuring airway inflammation and airway function. Mice will be used when we are investigating the effects on the immune system due similarities between human and mouse immune responses as well as the availability of cytokine assays and genetically altered animals. Rats will be used where a direct comparison is required with toxicological data to support decisions on how much substance to give to humans. Procedures will be subject to ethical review and are conducted by scientists with documented training and verification of competency. These scientists also have access to statisticians, Veterinary Surgeons and animal care staff for advice on study design and animal welfare matters.

	The majority of techniques (e.g. blood sampling, restraint and anaesthesia) used on these protocols are unlikely to cause adverse effects other than those that are mild and/or transient. Some techniques such as micro-organism infection studies may cause moderate adverse effects but humane study endpoints will be used to manage these at the minimum possible level.

Project 5	**Development of new therapies for diseases affecting the lung**	
Key Words (max. 5 words)	Inhalation, gene-based therapies, lung	
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)	There are many diseases that affect the lung for which there are no effective treatments. These include inherited diseases such as cystic fibrosis. The aims of this research are to develop and evaluate new therapies for currently incurable diseases of the lung.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We hope to develop new therapies, such as gene therapy for diseases such as cystic fibrosis and to learn how to use them most effectively and safely.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we aim to use approximately 100 mice per annum.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will be using wild-type mice and the methods of administration will involve inhalation, either by aspirated at the back of the throat or inhalation of aerosolized material. All substances will have been tested first on cells. We expect a low level of severity in these experiments. All mice will be killed humanely at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will use cultured cells for many experiments but the lung is a complex organ and there is no way to reproduce that complexity in a dish in the lab so live mice are necessary for these studies to have maximum scientific value.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We already have a lot of experience in these kinds of experiments. Power calculations will be used to predict number of mice required to obtain statistically valid date with the minimum number of mice
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.	Normal healthy mice will be used in these experiments, a widely used model in lung research. The methods of administration are minimally invasive with no surgery required. Mice will be anaesthetised for lung delivery to minimise stress and for methods of injection a pain killer such as lidocaine may be used. If there are any unexpected adverse events we will contact the designated vet.

Project 6	**Animal Models of Human Disease**	
Key Words (max. 5 words)	Animal models, new drugs, efficacy, safety	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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)	Overall Objective: To determine whether new drugs are able to prevent and/or repair tissue damage in animal models of lung disease. This objective will be achieved by:- • Performing studies to standards which ensure reproducibility and reduce bias. • To undertake model development when scientifically justified. • To use clinically relevant models, and endpoints, to help ensure translatability to specific human lung diseases including incurable diseases such as Idiopathic Pulmonary Fibrosis (IPF) and pneumonia.	
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 studies conducted under this project licence are intended to demonstrate whether new drugs work to prevent or cure human lung diseases for which there are currently limited treatment options (eg lung fibrosis) or for which current treatment options may not work in the future (eg infectious diseases). Studies will be conducted to standards associated	

	with regulatory drug safety assessment (ie Good Laboratory Practice standards or GLP). Studies conducted to GLP will ensure reproducibly by recording of all aspects of study conduct and comprehensive data collection on each individual animal in the study. These elements will help translation of the data for treatment of human diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Expect to use approximately 400 - 500 rats, mice or guinea pigs per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The work proposed in this licence involves the development of chronic models of disease eg lung fibrosis or pneumonia. Novel drugs will be administered to some groups of animals to determine if these novel drugs are able to prevent or cure the induced disease. For example, in models of lung fibrosis (or scarring) the novel drug may reduce the extent of scarring compared with disease control animals. In models of pneumonia, the novel drug may reduce the number of infections agents in comparison with disease control animals. The novel treatments may also improve the animals breathing and weight gain. However, development of pneumonia models – particularly chronic models, that best model human pneumonia, are challenging in animals. In general, animals are very good at clearing bacteria from their lungs without the bacteria causing an infection. However, some strains of bacteria can cause a chronic lung infection but a proportion of animals infected are likely to display signs which are life-threatening and these animals will be humanely killed. In addition, blood samples may be taken to in the animal models to determine the blood concentration

	of the new drug that is effective at either reducing fibrosis or infection. These data help 'translate' the effective dose range from the animal model to future clinical studies in humans
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The molecular and cell biology of how lung cells maintain air-filled sacs and prevent damage from environmental and infectious agents is not fully understood. For this reason, there are few effective drugs for life-threatening diseases such IPF. Currently, there are no in vitro models that model in all cellular and non-cellular elements of the air blood barrier, the immunity and nervous innovation of the lungs as well as the mechanical forces on the lung associated with breathing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal group sizes will be set using a combination of factorial analysis, data available in the literature and in-house/client experience. Advice may also be sought from in-house statistician for power analysis when the efficacy of a drug can be predicted from in vitro studies. Data collected on efficacy studies is likely to determine whether drug development is continued. Therefore lack of efficacy may reduce future toxicology studies. For some models, and study types, it may be possible to use the target tissue (eg lung) for more than one endpoint. This approach means that one animal is used instead of two.
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.	**<u>Choice of species</u>** The peer-reviewed literature demonstrates that rodent models of both fibrosis and pneumonia model many characteristics associated with disease in humans. For example rodent models of lung fibrosis were used to help develop the first marketed drug for lung fibrosis (eg pirenidone). **<u>Dosing, sampling and assessment</u>**

	All dosing, sampling and assessment procedures will be refined in accordance with published guidelines and internal experience, to minimise potential for animal suffering while ensuring appropriate conduct of experimental work. Specifically, dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm For protocols where severe signs may be anticipated, additional monitoring measures will always be applied during the periods of anticipated increased risk and humane endpoints applied as required. Unexpected, clinical signs which develop during a study will be assessed for their effect on the clinical condition of the animal. This may result in withdrawal of test item; humane killing of affected animals or contact being made with the Home Office to discuss whether the severity limits has been exceeded. **<u>Quality Control and Reproducibility</u>** Quality control and reproducibility of animal models will be continually reviewed by responsible persons. Positive treatment controls will be used in studies when available to demonstrate the model is reproducible (*eg* antibiotics in pneumonia studies). **<u>Preliminary Experiments</u>** The response of animals to a challenge agent cannot always be predicted from the literature. Therefore before starting any efficacy model, or changing an important experimental variable in new study (eg bacterial strain in the pneumonia model), preliminary experiments may be performed to assess the clinical response to the change.

Project 7	**Respiratory Pharmacology**	
Key Words (max. 5 words)	Respiratory diseases, lung, inflammation, rodent	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	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)	To help in the identification of new medicines for the treatment of human respiratory diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the UK, more than a quarter of people will die from a respiratory disease, various forms of which claim 70,000 lives a year. The figures put Britain at the bottom of the European league table in survival rates for illnesses such as asthma, influenza and chronic obstructive pulmonary disease (COPD). In addition these diseases place a significant the burden on society. The total cost of respiratory disease in the 28 countries of the EU alone amounts to more than €380 billion annually for treatment, lost productivity and disability costs. The aim of this project is to identify novel treatments for respiratory diseases that could be more effective than those currently available.	
What species and approximate numbers of animals do you expect to use	Mice 4700 Rats 4700	

over what period of time?	Guinea pigs 2200
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?	As we try to identify new medicines, studies conducted under this licence may induce some adverse effects in some of the animals. We need to induce some respiratory disease-like symptoms in order to allow the effectiveness of the potential new medicines to be tested. Typical adverse effects include a changes in appearance, for example ruffled fur or changes in behaviour, for example the animals may become subdued. Other effects may include reduction in body weight and/or reduced eating. The larger proportion of animals used in these studies will, however, not experience any noticeable adverse effects. For the vast majority of animals the severity level will be mild. However, as stated above in some studies the animals will experience some adverse effects but these would only cause the animal a moderate level of distress. In a few studies devices that allow the slow release of the new medicine may be surgically implanted under the skin under a general anaesthetic. At the end of the study the animals will be humanely killed. After the animals are killed samples of body tissue are sent to laboratories for close examination to give more information about the effects of the potential new medicines.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is a point in the development of new medicines when using cells alone or other non-animal experiments cannot reproduce what happens in the whole human body. Using isolated cells, cultured cells or tissue samples can mimic some aspects of the disease. It is extremely difficult, however, to do non-animal experiments that are able to predict how a potential new medicine will be distributed around a body and if it will have a specific adverse effect on certain organs of the body. To fully understand these different interactions/effects animals have to be used.

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

Project 8	**Airway biology**	
Key Words (max. 5 words)	Asthma lung inflammation therapeutics	
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)	Airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) are still a major challenge in the clinic, with moderate/severe patients still dying in significant numbers each year. We are looking for new mechanisms that could be involved in serious lung diseases, and testing hypotheses that modulation of these mechanisms could bring benefits to patients. In this process we will identify new therapeutic agents, which could be developed into new medicines.	
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 increase our knowledge of airway biology, including new immunological and physiological mechanisms involved lung diseases including asthma. We will identify new therapeutic agents which have the potential to become human medicines, bringing benefits to patients with currently uncontrolled disease.	

What species and approximate numbers of animals do you expect to use over what period of time?	We will mostly use mice, with the possibility of a smaller number of rats, in situations where mice are unsuitable. The total estimate over 5 years is 9960 mice and 840 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The overwhelming majority of animals will undergo lung inflammation or challenge models which mimic aspects of asthma, viral infection and other lung disease. These animals may experience reduced lung function due to mucous or swelling in the airways but are not expected to have any overt signs of respiratory difficulty and will likely fall into the Moderate severity band. A small proportion of animals (-5%) may undergo some surgical procedure. Some of these animals may have minor surgical procedures with minimal risks to welfare, and some animals may be used in experiments which are entirely under anaesthetic and from which the animal will not be recovered. A small number of animals may be used in surgical models where there is a greater risk of complication and lung impairment. Although most of these animals will be expected to reach only Moderate, it is possible that some animals may need to be categorized as Severe. At the completion of studies 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	Although more complex in vitro models of airway cells are being developed, it is still not possible to reproduce the complex interplay of immunological, neural and structural cells present in the living lung. We will always conduct as much work as we can in vitro before looking at in vivo systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental designs are reviewed by experienced. scientists, and the statistical methods we use to determine group sizes are reviewed by qualified statisticians.
3. Refinement Explain the choice of species and why the animal model(s)	Rodents, and mice in particular, are well characterised mammalian species with similar (but not identical) organ systems and immunological responses to humans. Most of the models we use are

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.	based on established methods which have been refined over many years, and where new models are derived, we take steps to ensure welfare is maximised from the start, so that the model has high standards built in from the beginning. All animals are purpose bred for scientific use and kept in state of the art facilities to keep them healthy and clean until use. Anaesthesia and analgesia are used in accordance with best practice guidelines to minimise any pain or discomfort during the scientific procedures.