Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 21

Projects with a primary purpose of Basic Research on the Respiratory System

Project titles and keywords

1. **Animal Models of Respiratory Disease**
    - Asthma, COPD, pulmonary fibrosis, IPF

2. **Genetic and cellular regulation of lung stem cells**
    - Lung; epithelium; stem cells

3. **Connective tissue repair, remodelling and fibrosis**
    - Scar, fibrosis, scleroderma, wound healing, vascular

4. **Regulation of inflammation, repair and tumourigenesis**
    - Fibrosis, inflammation, tumourigenesis, tissue repair

5. **Mechanisms of lung inflammation and hyperresponsiveness**
    - Inflammation, respiratory, allergic, non-allergic pharmacology

6. **New Therapeutics for COPD – III**
    - COPD, Tobacco Smoke, Pulmonary Inflammation, Mucociliary Clearance and Cough

<table>
<tr><td>PROJECT 1</td><td colspan="3">Animal Models of Respiratory Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Asthma, COPD, pulmonary fibrosis, IPF</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 Article 5)</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>
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<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 colspan="3">To identify effective new drugs for the treatment of chronic respiratory diseases such as asthma, COPD and pulmonary fibrosis (e.g. IPF). To achieve this objective some basic research is required to further our understanding of these diseases. Potential new drugs will be tested in rodents in which disease or aspects of disease have been generated. It is critical these rodent experiments resemble human disease as closely as possible, whilst keeping any distress or discomfort felt by the animals to a minimum. Therefore an additional objective is to optimise and refine these experimental systems.

Existing treatments for asthma (e.g. corticosteroids and bronchodilators) only relieve the symptoms of disease, without affecting disease progression. Furthermore, corticosteroids are ineffective in approximately 10% of asthma patients. The use of corticosteroids is also limited by undesirable side effects. Current treatments for COPD are largely</td></tr>
</table>

	ineffective and incapable of halting disease progression. There are no effective treatments available for fatal fibrotic conditions such as IPF. Therefore new, effective medicines are needed to treat patients with these severe 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)?	This project is expected to identify novel processes involved in respiratory diseases, such as asthma, COPD and pulmonary fibrosis, which will lead to new medicines to treat patients and improve their quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rodents (mice and rats) will be used in this project. It is anticipated that less than 1500 rodents will be used each year for the 5 year duration of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Reproducing aspects of human respiratory diseases may lead to those animals experiencing some signs of the diseases such as lethargy and breathlessness on exertion. In the majority of cases this is anticipated to give rise to no more than mild discomfort, with a small minority of animals experiencing moderate discomfort. Animals may undergo procedure involving injections. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences discomfort exceeding moderate. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon. At the end of each experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our current understanding of these respiratory diseases is insufficient to model the disease processes in their entirety, for the purpose of drug discovery, using cell based systems and/or computer models. These are chronic inflammatory

	diseases and thus, all components of an immune response must be present within the systems used to investigate novel drugs to better predict the effects of that potential new treatment in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant proportion of drug discovery is carried out using cells and cell lines (*in vitro*), with many thousands of potential drugs being screened to identify the most promising compounds. However, in order to study complex inflammatory responses, further testing is required in animals in which disease symptoms have been induced. To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity *in vitro* will be examined in animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the lowest animals on the evolutionary scale in which disease processes similar to human disease can be generated. As our understanding of human disease increases animal models will be continually reviewed to ensure that they are relevant to human disease. Selection of the most appropriate model to use to study a particular process in disease will be based on prior knowledge of that model. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences discomfort exceeding moderate. Humane endpoints are employed to limit suffering and burden to each animal. Substances given to the animals will be of a nature, route and frequency that of themselves will result in no more than transient discomfort and no lasting harm. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon.

PROJECT 2	**Genetic and cellular regulation of lung stem cells**	
Key Words (max. 5 words)	Lung; epithelium; stem cells	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 lungs are built and maintained by the actions of tissue-specific stem cells. Their behaviour must be controlled with exquisite precision so that they produce new cells of the correct type at the correct time in the correct place. Incorrect control of stem cell behaviour can be a contributory factor to several degenerative lung diseases and to lung cancer. The objective of the project is to advance our understanding of the regulation of lung stem cell behaviour. Specifically, we will study the regulation of lung stem cells by cell-to-cell signalling pathways and by their neighbouring 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)?	An improved understanding of the regulation of tissue-specific lung stem cells will advance the fundamental stem cell biology of the lung. In the long-term this work will also advance our understanding of human lung diseases. It will provide insights into the progressive development of these diseases and may suggest potential therapeutic strategies.	

What species and approximate numbers of animals do you expect to use over what period of time?	To breed multiple different strains of mice, carrying up to 6 different genetically-altered chromosomes, the project will require up 17,500 genetically-altered mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the animals Will be used in protocols under which they suffer either no, or very mild, adverse effects. For example, transient pain or discomfort following an injection. A smaller number of animals (<5% of the total) will be exposed to protocols of moderate or severe severity to model different aspects of human lung disease. The most severely-affected animals (<1% of the total animals in the project) might suffer weight loss (up to 20% total body weight), altered respiration rate and lethargy and some animals may be found dead. At the end all animals will be killed by a Home Office-approved method and their lungs collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Lung stem cells are regulated by their surrounding environment. This includes other lung cell types, nerves, blood vessels, immune cells from the circulation, and extra-cellular materials. This complex environment cannot yet be fully recreated in a dish in the laboratory and needs to be studied within the context of the whole organism. Nevertheless, some aspects of lung stem cell regulation can be studied in cultured cells and where possible we use this non-animal alternative.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform all preliminary experiments on lung stem cells which are grown in a dish in the laboratory. We use age-matched animals of the same background strain to minimise variability within each experiment and therefore reduce the numbers of animals required. We will also use statistical techniques to calculate the minimum number of animals which are required to obtain a conclusive result in each experiment.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	To study lungs in the context of the whole animal, we are restricted to studying an air-breathing vertebrate animal. We choose mice as a species which has lungs very similar to our own and is also easy to study and manipulate in the lab. The majority of our experiments are designed to study a small number of mutant lung stem cells in the context of a normal lung. This is a scientifically highly informative experiment which has the added benefit of being highly unlikely to have visible phenotypic consequences for the animal. Within this project, we will be studying up to 10 specific genes in animal lungs. Our project is designed such that these genes are studied in normal animals first. If the results suggest that the gene may have an important role in lung repair, or in human disease, these possibilities will then be investigated in injured cells, or injured lungs, growing in a dish in the lab (ex vivo). Only if these cell-based studies are scientifically useful will the genes again be studied in the whole animal, this time in the context of a more severe protocol which may cause some harm to the animals.

<table>
<tr><td>PROJECT 3</td><td>Connective tissue repair, remodelling and fibrosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Scar, fibrosis, scleroderma, wound healing, vascular</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</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>
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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>Connective tissue provides mechanical structure and organisation to all body structures and protects organisms from external threats and infection. Capacity for repair after injury determines the developments of disease and organ malfunction or failure. The processes governing this are not understood and will be defined through work proposed in this project.</td></tr>
<tr><td>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)?</td><td>Once the processes that determine the extent and quality of connective tissue repair and formation in normal growth and during aging are understood then many severe human diseases may benefit as there are currently no treatment to prevent or alleviate scarring and fibrosis after injury that is central to many common and uncommon diseases including lung, liver and skin fibrosis and cardiovascular diseases such as atherosclerosis</td></tr>
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	or pulmonary hypertension.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice provide a unique system to understand connective tissue repair and formation and explore key biological pathways especially as there are close similarities between human and mouse biology and mice have been characterised genetically and unique genetically modified strains are available. Approximately 750 to 1500 mice will be used in each of the major protocols for this project license although numbers will be minimised to that required for robust and reliable interpretation and analysis. Up to 1250 mice per year will be used in breeding for these experimental protocols.
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 used to provide samples for analysis of connective tissue in growth and development, including novel genetically modified (transgenic/knock out) animals. Experiments to assess response to tissue injury (e.g. skin biopsy) or therapeutic substances will be examined. This is classified as moderate severity. At the end of experiments mice will be humanely sacrified.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals, and specifically mice, provide the only way of studying connective tissue repair in vivo that permits detailed exploration of key pathways and mechanisms.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By using mice for development of parallel cell and tissue based experiments and through careful experimental design to minimise variability the number of animals used will be reduced to the minimum essential to provide reliable experimental results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mice provide a unique system because strains that reflect human diseases or have key targeted alterations in relevant biological pathways exists and can be used to test specific scientific questions. All procedures will be undertaken

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	within a well-managed and regulated animal facility by suitably trained staff and there will be close monitoring so that welfare cost are minimised.

<table>
<tr><td>PROJECT 4</td><td>Regulation of inflammation, repair and tumourigenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fibrosis, inflammation, tumourigenesis, tissue repair</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>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</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>The aims of this project are to further characterise the mechanisms involved in the regulation of inflammation, tissue repair, fibrosis and tumourigenesis. Through these studies we will work towards our overall goal of identifying novel therapeutic targets and treatments for chronic inflammatory and fibrotic diseases of the lung, kidney and liver, as well as, cancers of the lung and pleura.

This project focusses primarily on the lungs and pleural space, with relevant diseases including sarcoidosis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, obliterative bronchiolitis and emphysema, and tumours including mesothelioma and lung cancer. Chronic respiratory diseases are the third most commonly reported illness, affecting 7% of adults in the UK. They are a</td></tr>
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	major cause of morbidity and mortality and an enormous burden on health systems. Treatments for these diseases are, at best, limited or inadequate and, at worst, non-existent. The mechanisms involved in the development and progression of these diseases are incompletely understood. Tissue injury leads to the activation of the clotting cascade, as well as, resident tissue cells and recruited inflammatory cells with release of many regulatory chemical mediators. Under normal circumstances these wound healing processes are tightly regulated leading to restoration of tissue architecture and function. However, in disease states, there is aberrant or persistent inflammatory and/or wound healing in response to injury leading to the progressive development of excessive scar tissue and impaired tissue/organ function to a point where, in some cases, it may be incompatible with life. A better understanding of the mechanisms involved in the regulation of inflammation, tissue repair, fibrosis and tumourigenesis will facilitate the logical development of novel therapeutic approaches. The proposed studies should therefore further elucidate the mechanisms involved in the regulation of inflammation, fibrosis, and tumourigenesis, as well as provide proof of concept for novel approaches to the treatment of chronic fibrotic diseases and cancer.
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 project has excellent prospects of leading to an increase in our understanding of the mechanisms involved in regulating inflammation and scarring in normal and diseased organs, and in tumourigenesis. This will allow us to identify targets for the development of new treatments for fibrotic diseases and cancer. The project therefore has the potential to contribute to the development of life-saving and life-improving treatments for thousands of individuals.
What species and approximate numbers of animals do you expect to use over what period of	Mice are the species of choice because of the wide availability of well characterised models of injury and availability of genetically modified strains. However, occasionally when analytical reagents are not available, that are suitable for mice or when larger

time?	amounts of tissue are necessary due to detection limits of an assay we may use rats. We estimate that approximately 3400 mice and 300 rats will be required 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?	Many of the models of lung injury involve intratracheal (airway) instillation either of the initiating agent or agents to modulate gene expression or response to injury. This usually involves general anaesthesia and inhalation of the agent or injection of the agent through a fine tube inserted through the mouth into the trachea. These procedures cause only mild transient discomfort. Occasionally, intratracheal instillation may involve surgery to expose the trachea followed by direct injection. Animals will additionally receive one or more pharmacological or gene modulating treatments (either orally, intravenously, intraperitoneally or intratracheally). At the end of the protocol animals will be killed humanely in order to analyse the effects on the tissues. Where surgical procedures are used, animals will be given perioperative analgesics in order to control pain. In the worst case (less than 1%) there may be limited wound bleeding or wound infection may develop. Animals showing signs of these would be promptly treated or humanely killed. Agents used to induce lung disease are administered at doses below that which would be expected to cause respiratory discomfort or failure. Only handling discomfort and transient disorientation during recovery from anaesthesia are expected to be noticed by the animal. Any animals which become unwell (expected to be less than 1%) will be humanely killed. For the assessment of tumour growth animals are injected subcutaneously with tumour cells on the hind flank whilst the animal is under light general anaesthesia. The growth of tumours will be monitored and not allowed to exceed 2cm diameter. Animals will additionally receive one or more treatments (either orally, intravenously, or intraperitoneally). At the end of the protocol animals will be killed humanely in order to analyse the effects on tumour growth. Adverse

	effects are rare and animals generally show no signs of discomfort. However, occasionally tumours show signs of imminent eruption through the skin (expected in less than 1% of animals). Animals are monitored on a regular basis and if eruption appears imminent the animal will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research utilises multiple approaches including the use of patient cells and tissues and animal cell lines in which we undertake experiments to obtain proof of concept for our hypotheses prior to proceeding to the use of animal models to obtain conclusive evidence for the proposed concepts. The use of animals is necessary because *in vitro* systems cannot model the complexity of an organ such as the lung and its integration with the circulatory system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals used, proof of concept is obtained using *in vitro* experiments with human and/or animal cells and tissues. Inbred strains of animals are used wherever possible to reduce the effects of genetic variation and hence the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The animal models of disease used in this research have been chosen because they are recognised by those in the field to be the most appropriate available. We have extensive experience with these models, which we have refined over a number of years to allow use of the smallest species possible, reduce numbers of animals used and limit discomfort to a minimum consistent with a reproducible, statistically significant, pathologic outcome. We continually monitor and incorporate new approaches whenever possible to further refine, reduce and replace the use of animals.

PROJECT 5	**Mechanisms of lung inflammation and hyperresponsiveness**	
Key Words (max. 5 words)	Inflammation, respiratory, allergic, non-allergic pharmacology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to understand the role of different white blood cells which are part of the immune system in causing diseases like asthma and acute lung injury.	
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 anticipate that we will gain a greater understanding of the role platelets and other targets for the treatment of I respiratory diseases like asthma, This could potentially lead to the development of new drugs for this disease. Asthma currently affects 300 million people world wide, is a major cause of absence from work and is a substantial cost to the NHS. Chronic obstructive pulmonary disease will become the third leading cause of death by 2020 and accounts for 6% of the Health budget in Europe. Current treatments only modify the symptoms but not the causes of these diseases. Glucocorticosteroids are not very effective in chronic severe asthma or in COPD. There is therefore a need to find better drugs to treat the underlying cause of these respiratory diseases.	

What species and approximate numbers of animals do you expect to use over what period of time?	We will be using 16,000 mice of which 4000 have been allocated for breeding over 5 years and 1,000 rats in 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?	It is expected that no animal will experience more than moderate severity. The adverse effects will be controlled with special care measures and endpoints. Animals may undergo surgery and may experience pain but this will be controlled by providing pain relief before, during and after surgery. Some animals will be exposed to substances to cause inflammation in the lung or to prevent inflammation in the lung. We use non-lethal doses of substances and the animals do not experience difficulty in breathing, or feel unwell. In other cases we irradiate mice and this can cause the animals to appear lethargic but animals will be carefully watched and removed if any animal does not recover after a day. At the end of an experimental procedure or if it becomes necessary to alleviate suffering at any time then animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are interested in understanding the mechanisms that give rise to the physiological changes to the lung during inflammation. Whilst we can study individual cell types in culture there is currently no in vitro technique that can replicate the physiological changes in the way the airways constrict. Where possible we do undertake experiments using human cells in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are committed to maximising the amount of experimental information we obtain from each individual experimental animal, enabling us to hopefully minimise usage. We routinely use single strains of mouse in multiple research areas and use multiple organs from the same mouse in order to keep animal usage down. We are also trying to minimise the amount mice we breed for each research area.
3. Refinement	We are using mice as they have an immune system comparable in complexity to humans and are the

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.	most frequently used model for the human immune system. The use of genetically modified mice in our proposed program of work allows the maximum amount of experimental data to be obtained in the most efficient manner. We are mindful at all times to minimise welfare costs to the animals we use. All of our protocols are designed to minimise any welfare costs and in the event that it is unavoidable animals are monitored closely and welfare endpoints are rigorously applied, so that animals suffering is kept to a minimum.

<table>
<tr><td>PROJECT 6</td><td>New Therapeutics for COPD – III</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>COPD, Tobacco Smoke, Pulmonary Inflammation, Mucociliary Clearance and Cough</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
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<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>
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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>COPD is the third leading cause of mortality worldwide. The disease is characterised by pulmonary inflammation, cough, impaired mucous ciliary clearance, small airway remodelling, emphysema and reduced lung function. The predominant causative factor of COPD is the inhalation of tobacco smoke (TS). Currently there are no treatments that can stop the progression of the disease and the use of standard therapies such as inhaled corticosteroids (ICS) and bronchodilators have limited clinical efficacy. Furthermore, COPD exacerbations, defined as worsening of respiratory symptoms, often result in frequent hospitalisation, having a large impact on healthcare burden costs. Therefore, there is a high unmet medical need for novel effective therapies for this disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>The main aim of this project is to help identify novel treatments for COPD that can be progressed into clinical development. Specifically, the potential</td></tr>
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advanced or humans or animals could benefit from the project)?	benefits likely to derive from this project include identifying treatments that can reduce or stop the progression of pulmonary inflammation, cough, excessive mucus, reduced lung function and exacerbations in COPD patients. The ultimate aim would be to find novel therapies that would help reduce COPD associated mortality and morbidity.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a 5 year period the following number of animals may be used; • Mice – 20,000 • Guinea pigs - 2000 • Rats - 500
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?	Expected adverse effects associated with the licence include respiratory difficulties, laboured abdominal breathing, ataxia and potential loss of consciousness. The severity level for this PPL is severe. Animals will be humanely terminated by a Schedule 1 or non-Schedule 1 method and biological fluids, blood samples and tissue will be harvested for further analysis.
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 biological research when in vitro experiments cannot provide all the necessary conditions to further progress the development of novel treatments for human disease. In vitro models can mimic aspects of the pathophysiology of disease. They cannot, however, reproduce the complex interactions between different cells and mediators or reproduce the functional changes that occur as part of the ongoing disease process. It is only with in vivo experiments, that we can establish whether a novel treatment plays a functional role or is addressing a redundant pathway. Determining efficacy of novel substances in in vivo models is a prerequisite prior to testing in humans.
2. Reduction Explain how you will assure the use of minimum numbers	Statistical tests and power calculations will be conducted to ensure the minimum number of animals will be used to achieving meaningful results. Protocols and results will be reviewed to

of animals	determine whether fewer animals can be used to obtain meaningful data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats will be used as the lowest vertebrate groups shown to exhibit relevant COPD pathologies to appropriate stimuli. Genetically altered mice strains may be used, where appropriate, to aid target identification and validation. Guinea pigs will be used as their airway anatomy more closely resembles human and can evoke a cough response to appropriate stimuli, unlike mice or rats. Animals undergoing a regulated procedure are observed throughout the procedure. Any animals exhibiting changes in behaviour during recovery (such as isolation, lethargy) or appearance (piloerection, hunched posture) will be monitored at appropriate intervals (every 15 minutes) according to the nature of the response. Welfare assessment sheets (WAS) may be used to monitor individual animals. These sheets are based on a score system in order to determine a humane endpoint if necessary.