Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 4

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

Project Titles and keywords

1. **Polarity Regulators in Lung Development and Disease**
    - lung, repair, fibrosis, cancer, polarity
2. **Proteostasis in lung disease**
    - ER stress, cancer, pulmonary hypertensions

Project 1	**Polarity Regulators in Lung Development and Disease**	
Key Words (max. 5 words)	lung, repair, fibrosis, cancer, polarity	
Expected duration of the project (yrs)	5	
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
	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)	Lung disease affects a vast number of people worldwide. As an example asthma currently affects approximately 300 million people worldwide. In the UK alone respiratory disease kills one in four people. For many lung diseases there are currently no effective treatments available and there is a clinical need to develop new ones. This project has the following objectives: 1. To study how the lungs are formed- disruption to normal lung development can result in disease but first we must understand what normal lung is. 2. Many genes and proteins important for lung development also are involved in lung disease - once we identify factors important for lung development, we will investigate the role of these same factors in several lung diseases (Asthma, IPF, COPD and Cancer). 3. Kidneys develop by a similar mechanism to the	

	lungs termed branching morphogenesis and many of the same molecules are needed for both kidney and lung development. Where possible we will also investigate whether our genes/proteins of interest are also important for kidney development and adult kidney function.
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)?	Currently there are almost no effective treatments available for lung cancer, COPD or IPF. For asthma, steroid treatment to relive symptoms is effective for only a sub-set of patients (those with steroid responsive asthma) and there are no curative treatments available. This programme of work is designed to discover new pathways that can be targeted to provide completely new treatments for these diseases. In particular the aim is to discover molecules that can be used to repair and regenerate damaged lung tissue, a hallmark of all of the aforementioned disease. Because our approach focuses on finding targets to enhance tissue repair, any targets identified will likely benefit several if not all of these lung diseases. The aim of this PPL is to identify novel targets through discovery science which we will then validate using pre-clinical models such as human lung slices. On past experience we would aim to identify and validate up to 3 new targets within the next 5 years. The results can then be taken forward with new and existing collaborators within the pharmaceutical industry and/or existing technology transfer bodies to develop suitable therapies. This is likely to proceed outside of the timeframe of this PPL.
What species and approximate numbers of animals do you expect to use over what period of time?	We work with mice as a part of our research programme, combined with using human tissue, cell lines and available mouse and human bioinformatic data. Our studies require us to investigate the lung in vivo in a living animal. We work with mice because we need to look at whole intact embryonic lungs and to assess adult lung function in the presence of a particular genetic modification and critically mouse lung physiology and anatomy is similar to humans. Over the 5 years of this licence we may use up to

	15000 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the mice in this project (approx. 11,000) will be for breeding and maintenance and any adverse effects are unlikely. A small proportion of the GA mice may have a moderate phenotype such as those where breathing might be laboured due to lung abnormalities or those that in addition to lung abnormalities may also have other developmental anomalies such as head bobbing. In these cases the mice will be much more closely monitored and advice will be sought from the welfare officer (NACWO) regarding the animal's welfare. All phenotyping outlined in this licence will be moderate and mice will be closely monitored for any adverse affects. To enable us to identify novel information about mechanisms of lung disease and /or to identify potential new targets for treatment of these diseases we will may administer substances to induce lung disease including: House Dust Mites, Lipopolysaccharides, mould (Alternaria alternata), or bacterial or viral pathogens prior to testing the lung function of mice and them killing them to comprehensively assess the lungs. Because many of the same molecules are needed for both kidney and lung development and function, we may also analyse urine collected from these mice to assess their kidney function prior to killing them. To enable us to understand more about the mechanisms of lung cancer and/or to identify potential new therapies or biomarkers of lung cancer we may sensitise mice to develop tumours by examining whether lung tumours form in genetically altered mice with a mutation in one of our genes of interest that have been bred with mice containing an oncogenic Kras mutation (which sensitises them to developing tumours). We induce the Kras mutation in the lungs ONLY by combining a specific Kras mutant mouse strain with intra-nasal administration of the

	substance which can induce the Kras mutation (adeno-cre). These mice are then sacrificed at 6,12 and 20 weeks post adeno-cre administration. All mice are continually monitored for any adverse effects and all mice within an experiment are killed if any humane end-points are reached at any time-point. i.e. if a humane end point is reached at the 6 week time-point, the remaining mice killed and are not kept until the 12 or 20 week time point. Alternatively, genetically altered mice carrying a mutation in one of our genes of interest may be aged prior to assessing lung function and then killed or tumour formation assessed after mice have been killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary for us to work with living mice as lung function must be assessed where the whole organ is intact, contains all the normal cell types found in the lungs and is connected to an active circulatory system, this can only be done in an intact organism. Moreover our experiments require manipulation of the genome to understand gene function. The mouse shares similar physiology and anatomy to humans and in addition the mouse genome is known and genetic manipulation is possible in mice, mouse is therefore the best model system for these studies. The mouse is the lowest mammalian species in which the full range of genetic and physiological manipulations necessary for the investigation of lung development and disease can be achieved. It is critical to perform these studies in mammals since there are significant differences in the respiratory systems of frogs and fish. Where possible, aspects of these studies will be carried out using alternatives to live mice e.g. experiments in cell culture or using human lung slices. We will monitor any available and developing *in vitro* systems such as ‘lung on a chip’ and exploiting *in silico* technologies and databases where appropriate. We will continue to monitor and

	implement novel techniques using non-animal materials as they arise and will implement them where appropriate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Genetically altered (GA) mouse lines will only be maintained whilst there is a justified use for their continued breeding otherwise lines will be cryopreserved and removed from the shelf. Prior to establishing a new colony of GA mice under this licence, any available breeding data will be sought and well established breeding calculations will be used to predict output. In the case of lines of unknown viability, advice will be sought from experienced animal technical staff. For phenotypic analysis of GA mice. Where possible using existing data we have conducted statistical analyses to design robust experiments and minimise animal usage. Much of this work will be performed in collaboration with other laboratories using shared resources such as mouse lines and tissue therefore minimising the need to breed additional stock. Where possible, we will maximise the data obtained from each cohort of mice. For example, following lung function, before the conclusion of an experiment, urine may be collected to assess kidney function. When mice are killed, blood, bronchoalveolar lagage fluid (BALF) and any relevant tissues will be extracted from the same animals. Importantly, we will gain as much information as possible from each mouse and this will reduce overall the number of mice 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	The mouse is the lowest mammalian species in which the full range of genetic and physiological manipulations necessary for the investigation of lung development and disease can be achieved. Welfare assessment will be continually undertaken throughout the lifetime of the mice and where possible non-invasive phenotyping will be performed. We have introduced the following refinements to

(harms) to the animals.	minimise welfare costs to the mice: Non-invasive plethysmograph is used to measure lung function in some experiments as an alternative to restrained plethysmograph. The refined procedure involves placing the mouse into a chamber for a limited amount of time and allows free movement within the chamber. Sensitisation and treatments - only substances with previously proven use in sensitising or generating physiologically appropriate models of lung damage will be used. Where possible modification of genes is restricted to the lungs (our primary tissue of interest). This minimises any affects of the gene modification to one organ rather that the whole body. To induce lung tumours, we administer a substance directly into the lungs through the nose of the mouse (under anaesthesia). This restricts any tumour growth to the respiratory system.

Project 2	**Proteostasis in lung disease**	
Key Words (max. 5 words)	ER stress, cancer, pulmonary hypertensions	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The structure of each protein is critical to its function and is necessary for health. In many diseases, protein synthesis is defective and so proteins fail to 'fold' into their correct structures. The ability of the cell to respond appropriately to protein misfolding is called proteostasis (a contraction of 'protein homeostasis'). Much work, including my lab's, suggests that defective proteostasis is responsible for some lung diseases. Since drugs are being developed that affect proteostasis, it would be timely for us to examine how proteostasis might be targeted in lung disease to promote health. There are three mechanisms by which impaired proteostasis can affect tissues: (i) direct toxicity causing inflammation and cell death, (ii) altered cell division, and (iii) altered developmental signalling. We intend to address each of these aspects of proteostasis by examining models of lung disease: (i) lung cancer, which requires proteostatic systems to maintain tumour cell growth, and (ii) pulmonary hypertension, which involves abnormal development signals affecting blood vessels in the lung. We will divide this project into four key questions:	

	1. How do proteostatic signals maintain the health of lung tissues (airway lining epithelium and cells of the immune system within the lung)? 2. How do proteostatic pathways modify the development of malignancy? 3. How do proteostatic pathways contribute to vascular remodelling in pulmonary hypertension? 4. Is it possible to improve lung disease outcomes by using drugs that act on proteostatic signals?
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 has the potential to identify new drug targets for diseases including lung cancer and pulmonary hypertension. It may also identify drug targets to reduce acute lung injury following exposure to toxic fumes. This work is expected to help explain the mechanisms by which protein misfolding causes cellular damage and death in lungs diseases. We will identify new drugs targets to alter the response to protein misfolding, both to improve cell survival, for example during hypoxia, or to enhance cell killing, for example in the treatment of cancer. The scientific understanding provided by this project will benefit the wider research community and the pharmaceutical industry by providing novel targets for drug development. Patients may benefit in the short term through the identification of new tests for the severity of disease. Such tests could be used to provide more accurate prognosis or to identify patients requiring more intensive therapies. In the longer term (likely beyond the 5 years of this project), patients may benefit from more effective therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Our studies are restricted to mice. We plan to use no more than 11,750 mice. The actual number is likely to be much smaller.
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	The models of disease to be used in this project are classed as moderate severity: The cancer model we will use involves implantation of cancer cells beneath the skin on the animal's flank. These cells grow into tumours up to 1.2 cm. At this size the animal is killed. If tumours cause distress before this size, for example if the skin ulcerates, the animal will be killed.

at the end?	Rarely, the surgical procedures used to implant cancer cells or to implant intravenous catheters can cause mild pain, infection and the wounds can break down. Pain will be control with medication and infections treated with antibiotics. If these fail, the animal will be killed. Wounds that break open will be surgically closed if the University veterinarian considers this appropriate. Such surgery would be attempted only once per animal and if it were unsuccessful the animal would be killed. Housing the mice in low oxygen environments is well tolerated. The investigations of pulmonary hypertension require general anaesthesia. Genetically modified mice will be bred to determine if they are more or less susceptible to the models of disease under study. Most of these genetic modifications will not distress the animals. If severe pathologies were to develop in these genetically modified mice, for example the development of cancers, the animals would be killed before the disease could cause distress. At the end of each experiment the mice will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The studies planned follow on from much work in cultured cells and in *Drosophila* fruit flies. Unfortunately, these systems cannot faithfully predict the behaviour of a complex such as the mammalian lung.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Close working with statisticians will ensure accurate power calculations are performed. This will ensure that experiments use neither too many nor too few mice to arrive at a definitive answer. Our use of fruit flies for many early experiments (Replacement) will limit the numbers of animals required for the mouse investigations by helping to pre-select the most effective drug targets. Experiments will be performed in purebred strains of mice to reduce experimental variability. When particular stains of mice are not being used, we will stop breeding them and use frozen embryos or sperm to revive that strain only when required. We will use imaging techniques including echocardiography (heart ultrasound scan), CT scanning, and MRI scanning to allow

	measurement of cardiac function without the need for invasive procedures. Similarly, non-invasive tumour imaging will reduce the numbers of animals required by improving experimental reproducibility. Such imaging techniques allow repeated assessment of the same animal thereby reducing the numbers 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 mouse is the lowest animal in which lung function and growth can reliably be modelled. Collaborations are in place with experts in each mouse model to ensure state of the art experimental techniques are used: Non-invasive monitoring of pulmonary hypertension and cancer models using modern imaging techniques will allow animals to be followed over time reducing the numbers of mice needed.