



National Centre for the Replacement, Refinement
and Reduction of Animals in Research

List of abstracts for NC3Rs funded grants for 2007

Replacement of hamsters with physicochemical analytical methods for *Leptospira* vaccine batch potency testing

Dr N G Coldham, Veterinary Laboratories Agency (£181,072)

Aim

Leptospirosis is a potentially fatal bacterial infection transmitted from animals to humans. Nearly all mammals are capable of carrying the bacteria and, although the disease occurs in the UK, it is more prominent in tropical and subtropical regions. Vaccination is used to control the spread of Leptospirosis and each batch of vaccine is tested in hamsters prior to its use. An estimated 35,000 are used each year in the UK alone. The effects on the hamsters are severe as they must be infected with the disease and the potency of the vaccine is determined by the death of the animal. The aim of this research is to replace the use of hamsters in this test with a non-animal alternative.

Method

Recent technological developments in mass spectrometry can be exploited to enable the analysis of large biological molecules such as those found in vaccines. The protective components from virulent and avirulent *Leptospira* bacteria and a commercial vaccine preparation will be separated and detected with antibodies from vaccine immunised hamsters. Active components will be purified, identified and measured by mass spectrometry. This will enable the active components to be compared between batches of vaccines to ensure consistency. A similar approach has been attempted using antibodies to measure vaccine components but these are not widely available and the molecules they measure are poorly defined. In contrast, mass spectrometry is now widely applied across the biological sciences and such methods are transferable so components can be readily identified.

Implication for the 3Rs

The project will focus on finding a replacement for the test for the two component canine vaccine, which is considered to be particularly severe. If successful, this new approach to vaccine batch testing could replace

some of the 35,000 hamsters currently used in the UK each year. It could also impact more widely on vaccine testing for other vaccines.

Modelling the human asthmatic airway by tissue engineering

Professor D E Davies, University of Southampton (£299,875)

Aim

Asthma affects 1 in 5 children and 1 in 10 adults in the UK and even though animal models of asthma are routinely used, a paucity of new treatments have been developed since the 1960s. In this project, human airway cells grown in a culture dish will be used to mimic the asthmatic airway to replace current animal models and to potentially provide a better model of human asthma than currently available.

Method

Asthma tends to run in families, so animal models fail to mimic the interplay between environmental and genetic factors that cause human asthma. This project will use cells taken from the airways of asthmatic volunteers to develop sustainable, human-tissue based models that retain important genetic aspects of asthma and can be used in laboratory tests. The models will incorporate several different types of cells including epithelial cells that we already know respond abnormally to the common cold virus in asthma and dendritic cells that are involved in immune surveillance.

Implication for the 3Rs

This model of the asthmatic airway has the potential to replace the use of animals in testing new treatments for asthma.

A tissue engineering approach to reduce animal use in renal development and renal organ replacement technology

Professor J A Davies, University of Edinburgh (£364,044)

Aim

Kidney disease is a major cause of human suffering, with approximately 40,000 people in the UK seriously affected each year. An estimated 15,000 mice are used in the UK each year for research into kidney regeneration and transplantation. This project aims to produce cells that can be grown in the laboratory indefinitely, and can be combined to produce embryonic kidneys *in vitro* without any animals being needed.

Method

The research will produce cell lines that can give rise to specific components of the kidney and that can produce a whole embryonic kidney when used in combination. Based on existing systems, cells lines will be produced by immortalising cells from embryonic kidneys and by the sorting and culturing of the different types of cells that make up the kidney.

Implications for the 3Rs

Development of these cells will not only greatly reduce the use of animals in this type of research, but will also provide a system in which doing experiments is much quicker, easier and cheaper.

Metabolically competent stem cell systems: novel means to implement 3Rs in better drug safety assessment

Dr T Friedberg, University of Dundee (£323,624)

Aim

Developmental toxicology studies are carried out in animals to detect whether medicines are safe to be taken by pregnant women. Effects on development of the embryo and fetus are measured during these tests. Approximately 200,000 animals are used each year in Europe for this purpose. A test involving stem cells from mice embryos is currently used to answer some questions, but is not completely reliable because it cannot detect many drugs that have a smaller, but still significant toxic effect. Importantly, these tests do not recreate how the drugs would be metabolised and whether this would have any effect. The aim of this research is to 'humanise' this test to make it more predictive.

Method

The mouse embryonal stem cell test will be humanised by incorporating human drug metabolism by the addition of enzymes that are found in the liver. The project will also be employing stem cells from the human umbilical cord, which have the potential to be turned into different types of cell from the human body, to see how effective they are for testing reproductive toxicology.

Implications for the 3Rs

If the test can be made more relevant then this non-animal test has the potential to replace the use of more than 30,000 animals per year in Europe for embryotoxicity tests in the pharmaceutical industry.

Refinement of therapeutic intervention in a mouse model of amyotrophic lateral sclerosis

Dr A J Grierson, University of Sheffield (£164,760)

Aim

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is a neurological disease in which muscles waste away leading to loss of movement and basic functions like breathing, eventually resulting in death. There are approximately 5,000 sufferers in the UK and no known cure. Scientists can study mice in order to understand what is happening at the cellular and molecular level and new therapies are tested by how much they extend the lifespan of the mice. The mice experience substantial levels of suffering due to the progressive nature of the disease. The aim of the research is to refine this process to limit the suffering and potentially reduce the number of mice used in this research.

Method

A particular problem in treating ALS is to deliver therapeutic drugs to the motor neurons, which are buried deep within the spinal cord. The effects of drugs can be measured using fluorescent probes which are injected into the mice. This will enable direct measurements to be taken of how well the drug is working in motor neurons in the spinal cord of the mice. An obvious benefit of this is that the most effective drugs and the doses of them to use in experiments can be identified without having to let the mice develop paralysis.

Implications for the 3Rs

By studying the effects of drugs at an earlier stage, before the mice lose a substantial number of motor neurons and become paralysed, this research will refine the experiment and limit suffering. Equally important in terms of animal welfare, is the ability to exclude therapies that are ineffective at an early stage in the drug development process, thereby reducing the amount of animal testing carried out. On an estimate of 100 drugs being tested per year worldwide, the number of mice used would be reduced by 2,500.

Non-invasive identification of individual Xenopus by photography and image processing

Dr M Guille, University of Portsmouth (£59,208)

Aim

Over 10,000 frogs are used in the UK each year, often to produce eggs and embryos to study development. The aim of this project is to find a more humane way of identifying individual animals. Currently, techniques such as toe clipping, threading tags through the skin and implanting microchips are used but these are all harmful to the animals.

Method

Preliminary data suggests that individual frogs can be identified using digital imaging and computers. The patterns on the backs of the animals and the vein patterns on their feet can be measured. This research will develop the system to decide whether a single measurement or a combination is needed to identify the frogs and find out whether the markings change over time.

Implications for the 3Rs

An automated imaging system for identifying the frogs will be a refinement over the current identification techniques and reduce the level of suffering experienced by the animals.

An *in vitro* model of the human alveolus to predict the efficacy of systemic antifungal therapy

Dr W Hope, University of Manchester (£210,664)

Aim

The aim of this research is to develop an *in vitro* equivalent of the human lung for testing new drugs that aim to prevent potentially deadly fungal infections. The treatments being tested are for *Aspergillus fumigatus*, a fungus which causes devastating and frequently fatal infections in patients with damaged immune systems. There are estimated to be 4,000 cases in the UK each year and approximately half of patients will die before their immune systems can be restored through treatment. The development of any new anti-fungal treatments uses thousands of laboratory animals, mostly mice.

Method

The project aims to replicate the component of the human lung at which the fungal infection occurs - the alveolus. Human cells can be grown on a plastic membrane to simulate the alveolar wall, which normally serves as the barrier between inhaled air and circulating blood. Antifungal drugs will be injected into the *in vitro* model and their ability to treat fungal infection will be measured. Micro-pumps will be used to recreate levels of antifungal drugs observed in the blood of mice and humans in order to provide a more accurate representation of the differing conditions in these whole organisms.

Implications for the 3Rs

If successful, this *in vitro* model could significantly reduce the thousands of mice currently used to develop a new antifungal drug and largely replace the use of laboratory animals in this area of research.

Reduction, refinement and replacement of animal use by clonal sampling

Dr P Jones, Hutchison/MRC Research Centre (£235,096)

Aim

The skin of live mice can be used to study gene function and drug action but the side effects can include blistering, ulceration, tumours or other severe effects. This work will validate a novel approach to reduce the number of animals and refine these experiments so the animals suffer less.

Method

By genetically engineering mice it is possible to have the genes only expressed in 1 in 500 skin cells, rather than in all the cells, and for those cells to be labelled so the effects of the gene or drug can be studied. Hundreds of labelled cells can be surveyed from one mouse after it has been humanely killed. By using mathematical analysis it is possible to work out how the cells are behaving with just 10-20 mice and predict what will happen after many months with just a few weeks of treatment.

Implication for 3Rs

Because the gene is expressed in only 1 in 500 cells, the surrounding normal cells are able to keep the skin healthy, which refines the experiment and greatly decreases the chance of suffering being caused to the animal. Running the experiment for weeks rather than months also minimises the time the animals are exposed to the treatment. A significant reduction can be achieved because 20-30 animals are needed per gene or drug, compared to the 200 or more currently used. The researchers also plan to use the latest tissue culture techniques to grow human skin cells and use mathematical analysis to see if these could replace animals in this type of experiment.

Pseudoislets as a model system to study beta cell dysfunction in diabetes

Professor P M Jones, King's College London (£387,732)

Aim

Over 2 million adults in the UK have diabetes, plus an estimated 750,000 unaware that they have the disease. The aim of this project is to grow islets from the pancreas in order to understand the biology of the cells that contribute to diabetes. Currently, the most common source of islets for these studies is laboratory animals, particularly rats and mice, and it is estimated that tens of thousands of these animals are used each year. This project proposes to develop an alternative to taking islets from animals.

Method

There are already a variety of animal-derived hormone-producing cells that have been modified to grow continuously in tissue culture without the need for using more animals. However, islets are complicated structures in which interactions between cells are important for function. When the cells are grown in isolation they no longer behave normally, and so their usefulness for experiments is limited. The interactions between islet cells have already been investigated to find out how to put the cells together to form islet-like structures (pseudoislets) which behave in a similar way to real islets and produce insulin. The current pseudoislet model is much better than the original cell lines, but it is not yet as good as a real islet. This research will improve the function through a further series of experiments.

Implications for the 3Rs

If the pseudoislets can be improved then this model would replace the use of an estimated 30–40,000 animals a year that would otherwise be the source of islets. This could substantially reduce the numbers of animals used in diabetes research.

Humane endpoints for rodenticide testing

Dr A MacNicoll, Central Science Laboratory (£63,780)

Aim

Rodenticides are a category of pest control chemicals intended to kill rodents and they are tested on rodents to see if they are effective. The project aims to identify biological markers from blood, faeces and urine, which can predict whether a rodent will die before the onset of suffering rather than waiting for death to occur.

Method

There is a lag-time, of 4-6 days, from ingestion of bait to death with all anticoagulant rodenticides. Behaviour of rats will be observed before and after the poison is given to them. Corticosteroid levels will be monitored as an indicator of stress and a range of vitamin K-dependent blood clotting proteins will be measured. Other techniques will be used to identify changes in the biochemistry of blood, faeces and urine. The results will then be used to identify which factors can be monitored, preferably through non-invasive procedures, to predict death and survival of rats after exposure to anticoagulant rodenticides.

Implications for the 3Rs

Being able to humanely kill the animals instead of waiting for death to occur will significantly refine a procedure of substantial severity so that the animals used experience less suffering.

Development of a reduced severity rat epilepsy model

Dr G Woodhall, Aston University (£152,048)

Aim

Epilepsy affects between 1 and 2% of the world population, causing considerable suffering to patients. Most animal models of epilepsy rely on the animals undergoing continuous seizures lasting longer than 30 minutes. One such model has provided insights into how epilepsy changes the structure and function of the brain, but this model can cause the death of animals through uncontrollable epileptic seizures. The research proposed here is aimed at reducing the chance of uncontrollable seizures, which means that fewer animals will be needed for this research and that there will be less suffering.

Method

A chemical called dopamine has a role in the control of seizures through its actions on the brain. This research aims to take advantage of this mechanism to try and minimise the chance of an uncontrollable seizure and to reduce seizure activity to the minimum level possible.

Implications for the 3Rs

Currently this procedure can cause substantial suffering in some animals and this can be minimised by the refinement of the test. The numbers of animals needed should also be reduced as more data can be collected from fewer animals.