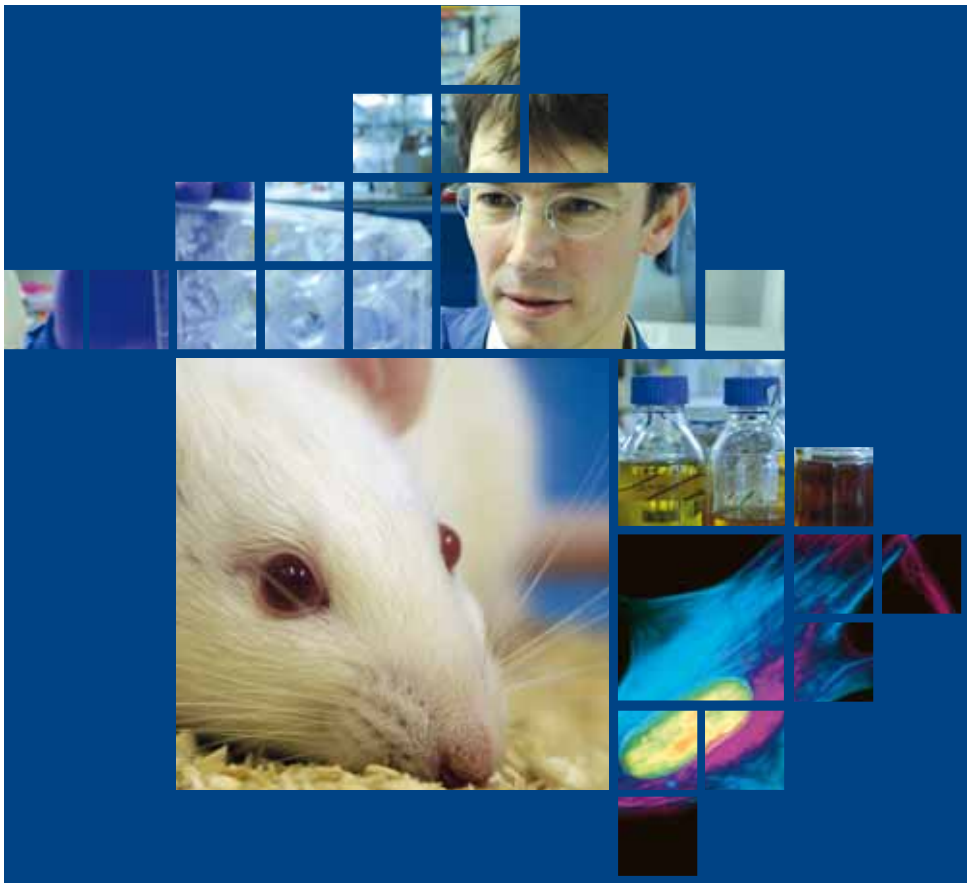
NC3Rs

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



CHANGING THE LANDSCAPE

SCIENTIFIC INNOVATION AND THE 3RS

Annual Report 2009

THE 3Rs

Replacement refers to methods that avoid or replace the use of animals defined as 'protected' under the Animals (Scientific Procedures) Act 1986.

Reduction refers to methods that minimise animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing future use of animals.

Refinement refers to improvements to husbandry and procedures that minimise pain, suffering, distress or lasting harm and/or improve animal welfare.

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FOREWORD

2009 has been another very productive and successful year for the NC3Rs, with the Centre continuing to make a significant impact on the scientific landscape, both nationally and internationally.

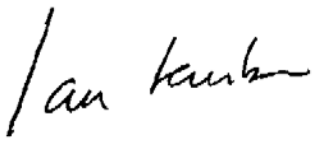
During the last 12 months, the 3Rs have been high on the scientific and political agendas with the 50th anniversary of Russell and Burch's publication *'The Principles of Humane Experimental Technique'* and the proposal from the European Commission for revisions to Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. In responses to the proposed revisions there has been widespread support for our work, with organisations from within and outside the UK citing the innovative approach of the Centre as a model for promoting the 3Rs.

The use of non-human primates (NHPs) is perhaps the most controversial area of the draft revised Directive. There has been much debate about the use of NHPs over recent years, with some groups calling for a ban on their use. Against this background, we have worked closely with the pharmaceutical and biotechnology sectors, and with international regulatory agencies, to identify novel approaches that will minimise the use of NHPs, even in complex areas such as the safety assessment of biopharmaceuticals.

Our Centre-led programmes have continued to provide an important and unique platform for critically evaluating animal models and potential alternatives, facilitating data sharing, and fostering partnerships and networks across the biosciences sector. Over the last 12 months, the Centre's scientific staff have published an impressive 16 papers deriving from programmes they lead on a wide range of topics from tissue engineering to redundancy in acute toxicity testing of chemicals (see page 21).

During 2009, there has also been a formal five year review of the NC3Rs undertaken for the Department for Business, Innovation and Skills. This was conducted by an expert panel, chaired by Sir Kenneth Calman, which considered our past performance, future plans and the continued need for the Centre. The final outcome will be shared with our partners as soon as the review is complete.

Many individuals, groups and organisations are involved in the work of the NC3Rs and this is a good opportunity to express our thanks for the support and time provided during 2009. This of course extends to those who fund the NC3Rs and it was welcome news that the chemical industry agreed in 2009 to provide renewed funding for a scientific post at the Centre.



Professor Ian Kimber, Chairman

CELEBRATING THE 50TH ANNIVERSARY OF THE 3RS

2009 marked the 50th anniversary of '*The Principles of Humane Experimental Technique*', the book written by William Russell and Rex Burch, which first described the 3Rs as an ethical framework for conducting research using animals. The NC3Rs used this important milestone as an opportunity to expand its public engagement activities, focusing on audiences with an interest in science but no specialist knowledge of the 3Rs.

The NC3Rs has increased the breadth and scale of 3Rs information available to MPs.

Animal research is one of the most contentious issues in science. Opinion polls on public attitudes suggest people often hold conflicting views simultaneously and a more nuanced position than the polarised debate often presented. Since 1999, Ipsos MORI has conducted a series of public opinion polls on animal research and these provide the most reliable benchmark available on trends in attitudes. The most recent survey results[1], carried out for the Department for Business, Innovation and Skills, show that approximately three-quarters of British adults conditionally accept the use of animals as long as it is for medical research purposes, there is no unnecessary suffering, and there is no alternative. Nevertheless, public concern is significant with nearly a third of British adults objecting to experiments on animals.

The 3Rs links science and society because it provides a framework for balancing scientific innovation with the ethics of animal research. It is important to increase opportunities for public engagement on the 3Rs to improve understanding and promote informed debate. As a first step to achieving this the NC3Rs organised a varied programme of events during 2009. This included an essay competition run in conjunction with *New Scientist* magazine, a panel discussion at the Cheltenham Science Festival, and a poster event held at the Houses of Parliament.

Showcasing UK 3Rs activity

Parliamentary interest in animal research is high. Over the last five years, the number of parliamentary questions on the use of animals in scientific procedures has doubled; in 2009, a quarter of those relating to animal use referred specifically to the NC3Rs. Since 2004, there have also been 39 Early Day Motions (EDMs) on animal research, and during the parliamentary session 2008/9, EDM 545 on 'Scientific procedures on animals and the use of alternatives' received the fifth highest number of signatures of all EDMs tabled.

The NC3Rs has increased the information available to Members of Parliament (MPs) on the breadth and scale of 3Rs activity ongoing in the UK. In March 2009, the Centre held its second poster event in the Houses of Parliament to highlight the latest 3Rs research to parliamentarians and invited guests. The event, titled '*The 3Rs today*', was sponsored by Lord Sainsbury, the former Minister of State for Science and Innovation who established the NC3Rs. It was attended by 30 MPs and peers, and more than 100 representatives from the many organisations the Centre works with.

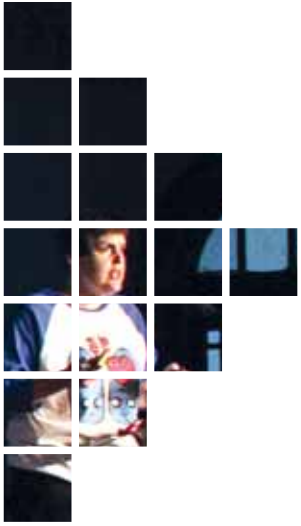
Forty-three posters describing research projects across all three 'Rs' were presented by scientists from academia and industry. A prize of £1k was awarded for the best poster for each 'R' based on the quality of the science, the 3Rs impact, and the ability of the poster presenters to communicate their research to a non-specialist audience. The prizes were awarded by Lord Drayson, Minister of State for Science and Innovation, to the following researchers:

Replacement: Dr Deborah Holliday, University of Leeds – A 3D model of breast cancer: towards replacing the need for animal experiments

Reduction: Dr Steven Wang, AstraZeneca – Reduction in an animal model used in obesity research

Refinement: Mrs Cerys Lovatt, GlaxoSmithKline – Opportunities for refinement and reduction using dried blood spots for generation of toxicokinetic data.

1. Ipsos/MORI poll (2009) Views on animal experimentation.



The NC3Rs/New Scientist essay competition attracted more than 120 entries from all over the world.

A future beyond animal research

In March 2009, the NC3Rs launched an essay competition with *New Scientist* magazine entitled '*Beyond animal research*', which challenged its nearly 200,000 print readers and three million monthly web visitors, to think imaginatively about a vision for the future where fewer animals are needed for basic and medical research. A judging panel, including the eminent scientists Professor Lewis Wolpert, University College London, and Professor Dame Nancy Rothwell, University of Manchester, and chaired by the editor in chief of *New Scientist*, Mr Jeremy Webb, considered more than 120 entries from all over the world. The winning entry entitled '*Your virtual twin*' (see opposite), by NASA scientist Dr Natalia Alexandrov, was published in *New Scientist* in June 2009. This, and the two runner-up essays, also appeared on **NewScientist.com**.

Cheltenham Science Festival event

Building on the theme of the essay competition, the NC3Rs and *New Scientist* also hosted a joint event at the Cheltenham Science Festival – widely regarded as the UK's premier public science event. This was the first time the Centre had hosted a public debate. Attended by 80 members of the public and chaired by *New Scientist* editor, Dr Roger Highfield, the event featured Dr Kelly BéruBé, a cell biologist from the University of Cardiff, describing her work using an *in vitro* "micro-lung", Dr Steven Manos from the Centre for Computational Science at University College London talking about computer simulations of the human body, and the science fiction writer Dr Paul McAuley communicating his vision on the future alternatives to using animals. A lively discussion followed the talks, with questions from the audience on the extent of scientific engagement in the 3Rs and the wider ethical issues of using animals in research.

YOUR VIRTUAL TWIN

Winner of the NC3Rs/ *New Scientist* essay competition

A quiet night in December 2050. Small town anywhere on Earth. In the hospital, little Peter has just made his appearance in the world. As the happy family takes pictures of mother and baby, the thought that his birth is incomplete is far from their minds. And the birth will not be complete for a few hours, until hundreds of miles away his virtual twin is born.

While Peter is dreaming his first dreams, samples of his blood and tissue are analysed and the resulting data transmitted to the simulation and modelling department of the regional medical centre.

It is expensive and time-consuming to build a virtual human from scratch, so a library of averaged mathematical models of newborns is maintained. The initial models differ by sex, ethnicity, geographic origin, basic genetic make-up and other salient characteristics.

When Peter's data arrive, they are integrated with a model whose attributes most closely resemble Peter's. The vast resulting computational model is as unique as Peter himself.

Throughout Peter's childhood his parents take him for medical check-ups, inoculations and treatment for a broken arm and colds. All this information makes his virtual twin grow. When the boy is 10, flu leaves him with a complication - severe bronchitis. Which antibiotic to prescribe? The family doctor downloads Peter's virtual twin, updates it with the latest tests, and runs simulations for the range of available antibiotics to anticipate short-term and long-term effects. This identifies both the perfect drug and one that would have caused a life-threatening, long-term effect on Peter's blood-clotting ability, possibly leading to a future stroke.

Why an individual twin? The laboratory animals used to develop drugs do not always represent humans adequately, and even well tested drugs can cause adverse reactions in some people.

How far are we from building useful and practical virtual twins? There are many sophisticated models of individual organs and systems, such as network models of the metabolic, immune, nervous and circulatory systems; computational fluid dynamics models of blood flow; structural models of the heart and other muscles. These models will continue to grow in fidelity, but the behaviour of a complex system is a function of its complexity, and the biggest barrier is, arguably, the integration of subsystem models into one systemic model.

As well as the difficulty of modelling every aspect of the organism in terms amenable to computation, the challenges are in combining disparate models and the sheer volume and complexity of the information needed. However, in this writer's opinion, the situation is by no means hopeless. Moore's law of growing computational resources will provide the necessary storage and speed. The complex mathematics involved will be more challenging, but by no means prohibitively so.

The benefits of a virtual twin that evolves along with the human it represents would be enormous. The action and long-term consequences of most drugs are at present a mystery, despite careful research and animal testing. We cannot quantify the processes actually taking place in our bodies as a result of an illness or in response to a drug. A faithful computational representation of an individual would yield quantifiable, rigorous measurements of the model's reactions. There would no longer be a need to identify a population for a drug trial, nor would years go by in tests while desperate patients are waiting for a new drug. A set of virtual patients with precisely known characteristics could be selected at any time, and drug research conducted at computational speeds, resulting in safe, effective treatments based on individual patients' characteristics.

This is the future of virtual twins. And no more animal testing.

Dr Natalia Alexandrov, NASA

DEVELOPING NEW RESEARCH TOOLS AND MODELS

The NC3Rs continues to position the UK as a global 3Rs powerhouse by funding research to develop new models and tools, increase knowledge transfer across scientific disciplines, and invest in research careers. The Centre now has three schemes: the main 3Rs Research Funding Scheme, which awards grants of up to three years, the Small Awards Scheme, and a new PhD Studentship Scheme which was introduced in 2009.

Capacity building and career development in the 3Rs

The NC3Rs Studentship Scheme was launched as part of a long-term strategy to develop a cohort of young scientists from all areas of research with an awareness of the 3Rs from the early stages of their careers. Other organisations, including the Association of the British Pharmaceutical Industry (ABPI) and the Biosciences Federation, have also highlighted the need for more PhD training opportunities with a 3Rs focus. Forty-eight applications from 27 UK research organisations were received, and five awards were made to leading research groups (see opposite). All applications were subjected to peer review, with funding recommendations made by a newly-appointed panel, chaired by Dr Malcolm Skingle CBE from GlaxoSmithKline (GSK) (page 28). The awards of £30k per year cover the student stipend, fees and research costs. The aim is to increase the number of studentships available as the scheme develops.

Mr Goncalo Leite

STUDENTSHIPS AWARDED IN 2009

The new NC3Rs Studentship Scheme will influence the training and development of tomorrow's research leaders.

Professor Hannah Buchanan-Smith, University of Stirling

Refining rearing practices in marmosets

Improvements in the diet of common marmosets bred for research has led to an increase in litter size from two to three or even four infants. This increase is associated with high infant mortality, birth complications and rearing practices that involve temporarily removing one or more infants from the family unit, which is known to affect behaviour and physiology. This project will compare the survival, development, behaviour and welfare of marmosets reared under different husbandry practices to identify the best breeding and rearing methods. The findings could have far-reaching implications for the welfare of marmosets in breeding and research facilities, and for the reliability of the marmoset as a model in research and testing.

Professor Jane Hurst, University of Liverpool

Taming anxiety and variation in laboratory mice

This project aims to establish easily-implemented, practical approaches to reduce the anxiety and stress responses that have been shown to occur in laboratory mice during routine handling, restraint and scientific procedures. Refining these procedures will improve the welfare of laboratory mice throughout their lives. It may also reduce data variability and the confounding effects of stress responses in experiments, helping to reduce the number of animals used and increase the reliability of comparisons between experiments and laboratories.

Dr Gareth Jenkins, Swansea University

Validating defined genotoxic thresholds, leading to better *in vitro* risk assessments of carcinogenic potential

All new chemicals are tested for their potential to cause DNA damage (genotoxicity), as this is linked to cancer. A tiered approach is used, with the chemicals first being tested *in vitro*. Those that induce DNA damage (test positives) are then usually tested again using animals. Some genotoxic agents give positive results *in vitro* at relatively high doses, but do not cause DNA damage at low doses more typical of human exposure levels. Identifying the dose below which DNA damage does not occur is important because understanding how this relates to human exposure levels can provide an opportunity to avoid using animals.

This project will use an *in vitro* system to assess the genotoxic potential for a wide range of chemicals, helping to establish optimum testing strategies for evaluating the effects of dose and potentially reducing animal use.

Dr Donna MacCallum, University of Aberdeen

An *in vitro* model system to assay kidney-pathogen interactions determining outcome of *Candida albicans* infection

The mouse intravenous challenge model is currently used to assay the virulence of *Candida albicans* strains. The main measures of virulence used are time to death or fungal burden in the tissues of mice killed at specific time points post-infection. The aim of this project is to develop an *in vitro* virulence model using primary kidney cells or established cell lines. The new *in vitro* model will be used to evaluate the early stages of systemic infection in the kidneys, potentially replacing the use of mice in these studies.

Dr Matthew Wright, Dr Fiona Oakley and Professor Derek Mann, Newcastle University

Applying the 3Rs to liver fibrosis research

This project aims to reduce and refine the use of mice in two aspects of liver fibrosis research. Bile-duct ligation, a common surgical technique for generating periportal liver fibrosis in animals, often gives rise to a high level of mortality. By using the chemical methapyrilene to cause periportal liver damage instead, the severity classification of the procedure under the Animals (Scientific Procedures) Act 1986 (ASPA) could be refined, from 'substantial' to 'moderate'. In addition, the project will investigate real-time imaging of the progression of liver fibrosis, using antibodies to detect myofibroblasts (the fibrosis-causing cells of the liver). This will allow repeated readouts from the same animal to be taken, thus reducing the numbers of animals used.

With £12.5 million committed to-date, the NC3Rs is the largest funder of 3Rs research in the UK.

Research funding

In 2009, the Centre awarded 13 grants totalling £4.5 million (see opposite). Four of the awards will use stem cell technologies to explore opportunities for reducing or replacing animal use. Of these, two have implications for reducing the use of genetically modified (GM) mice, where the largest increase in numbers has been seen in recent years. Of the remaining awards, three have been made to scientists previously funded by the NC3Rs (Emerson, Perry and Roughan) and build on the progress already achieved.

With £12.5 million committed to-date, the NC3Rs is the largest funder of 3Rs research in the UK, supporting research across a range of disciplines, as shown below. Over the next 12 months, the NC3Rs will be reviewing its research portfolio and identifying further opportunities for maximising the dissemination and uptake of the output from previously funded work.

The 3Rs Research Funding Scheme funds hypothesis-driven and applied research. All applications to the Scheme are subject to a peer review process using the Medical Research Council (MRC) scoring criteria to ensure that the work the NC3Rs funds is of a comparable standard. In 2009, Professor Sir Andrew McMichael, University of Oxford, was appointed as the new Chair of the Grant Assessment Panel (page 28). Over the last five years, the research funding scheme has attracted an increasing number of applications (see below). With an award rate of 18%, the Centre is funding at a rate comparable with the UK's major biosciences funders.

The NC3Rs continues to drive the 3Rs agenda by identifying research priorities to stimulate applications in areas of particular concern for animal welfare or where there is an opportunity for greater exploitation of new technologies or model systems.

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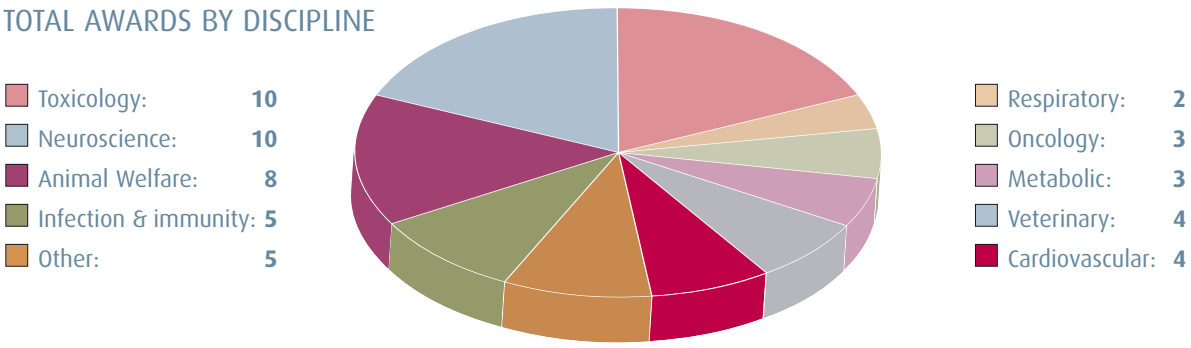
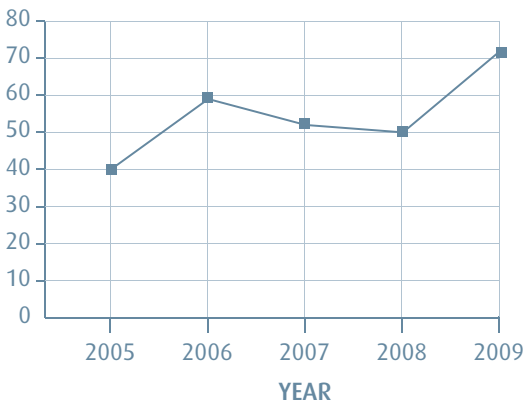
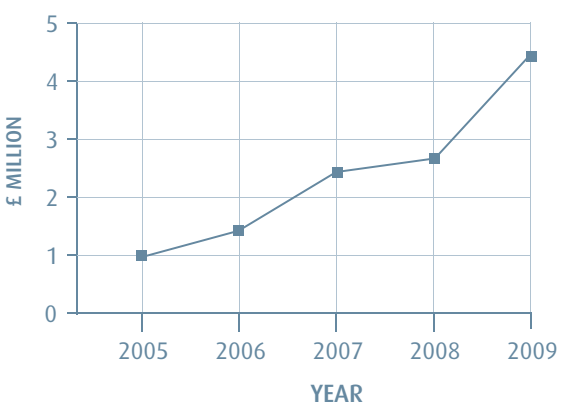
TOTAL AWARDS BY DISCIPLINE

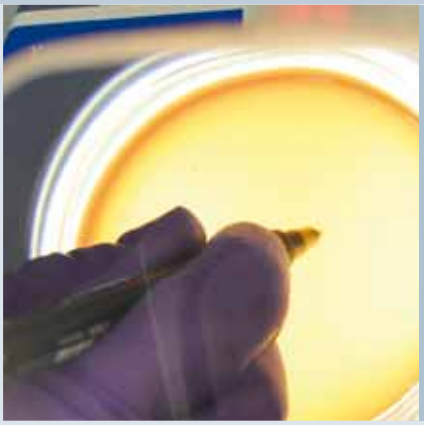


NUMBER OF APPLICATIONS



TOTAL AWARDED





RESEARCH GRANTS AWARDED IN 2009

Professor Qasim Aziz, Queen Mary, University of London (£287,604)
Refinement of animal studies on emesis by defining human biomarkers of nausea

Dr Michael Emerson, Imperial College London (£188,290)
Reducing and replacing mouse use to model the human platelet response *in vivo*

Dr Berthold Gottgens and Dr Aileen Smith, University of Cambridge (£355,688)
Transgenic ES cell differentiation systems to replace transgenic mouse analysis of tissue-specific regulatory elements

Dr Majid Hafezparast and Dr Timothy Chevassut, University of Sussex (£216,696)
Development of induced pluripotent stem (iPS) cells from mutant mouse models in order to reduce animal use

Dr Susan Jobling and Dr Edwin Routledge, Brunel University, and Dr Catherine Jones and Dr Leslie Noble, University of Aberdeen (£544,508)
Of molluscs and men: the snail assay as an alternative to the Hershberger male rodent assay for the detection of androgens and anti-androgens

Professor Mark Lewis, University of Bedfordshire, and Professor Linda Greensmith and Dr Vivek Mudera, University College London (£403,876)
Engineering fully functional, integrated skeletal muscle

Professor Ian Mackenzie, Barts and The London School of Medicine and Dentistry (£345,092)
Development and validation of *in vitro* methods for assaying cancer stem cell responses to therapeutic challenge

Dr Kevin Moffat and Professor Bruno Frenguelli, University of Warwick (£370,296)
Drosophila as a model for Alzheimer's Disease

Professor Hugh Perry and Dr Tracey Newman, University of Southampton (£210,884)
A compartmentalised chamber for the *in vitro* study and manipulation of axon degeneration

Dr Johnny Roughan and Professor Paul Flecknell, Newcastle University (£380,748)
Assessing the welfare of mice used in cancer research

Dr Vasanta Subramanian, University of Bath (£297,356)
iPS cells from Amyotropic Lateral Sclerosis (ALS) patients – towards replacing animal models for ALS

Professor Susan Watson, Dr Richard Argent, Dr Anna Grabowska, Dr Rajendra Kumari and Dr Snjezana Stolnik-Trenkic, University of Nottingham (£407,256)
The human tumour micro-environment modelled in *in vitro* biomatrices and applied to cancer and drug discovery

Dr Robin Williams, Royal Holloway University of London and Professor Matthew Walker, University College London (£415,248)
Replacing, refining, and reducing animal use in epilepsy research using a non-sentient model

Together with the Biotechnology and Biological Sciences Research Council (BBSRC), one priority area was highlighted for 2009: '*Replacing animals protected under the ASPA with invertebrate models*'. This priority reflects the potential opportunities in many areas of science for using less sentient lower organisms to replace the use of vertebrates (referred to as relative replacement). Two applications were funded within this area by the NC3Rs and one by the BBSRC.

One of the projects awarded by the NC3Rs under the 2009 priority area will investigate the use of the tropical freshwater snail (*Biomphalaria glabrata*) as an alternative to castrated rats in the Hershberger assay. This assay is used to assess the androgenic and anti-androgenic properties of chemicals and typically uses 30-50 animals for each test. It is the gold standard pre-clinical test used in the development of pharmaceuticals to treat hormone-dependent male cancers and is currently being considered by international regulatory bodies as a screen for endocrine disruptors. The idea that the snail can be used as an alternative to the rat is supported by the many features of its reproductive system, from the molecular level to gross anatomy, which it shares with mammals. This project will compare the effects on the snail reproductive system of a battery of known androgenic chemicals, with the responses observed in mammals.

The other project awarded by the Centre under the priority area will use the fruit fly (*Drosophila melanogaster*) instead of GM mice to study Alzheimer's Disease. Many GM mouse models of Alzheimer's Disease have been generated, and since the disease is a progressive condition it is often necessary to follow the mice for extended periods of time until some form of debilitation is evident. This can have animal welfare implications and is compounded by the difficulty in generating animal models that mimic all aspects of the human disease. It has previously been shown that human genes involved in the pathogenesis of Alzheimer's Disease can be expressed in fruit flies. This project will investigate using fruit flies to explore novel biochemical pathways involved in the disease and for high throughput screening of new drugs.

Small Awards Scheme

In partnership with the Laboratory Animal Science Association (LASA), the NC3Rs runs the Small Awards Scheme. This scheme is open to scientists, veterinarians, and animal technicians for small-scale research projects and training. In 2009, in response to feedback from applicants, the award value was increased to £3k. From 20 applications, seven awards were funded, covering a range of disciplines from optimising techniques for the non-invasive measurement of stress hormones in rats, to creating a reference catalogue of ultrasound images of mouse development *in utero* (see **www.nc3rs.org.uk/fundedsmallawards** for the complete list).

MINIMISING ANIMAL USE IN VETERINARY RESEARCH AND VACCINE TESTING

More than 10,000 animals were used for applied studies in veterinary medicine in Great Britain in 2008. Funding from the NC3Rs is generating new tools for basic research and regulatory testing that will minimise animal use in veterinary science.

Developing an air-interface *in vitro* organ culture model of bovine respiratory epithelium

In 2009, NC3Rs grantholder Dr Dan Tucker, and his colleagues at the University of Cambridge, published work on the development of a new *in vitro* model for studying infectious respiratory diseases of cattle, which are major causes of animal welfare and economic concern globally.

Bovine herpesvirus (BHV-1) respiratory infections are widespread. BHV-1 infection alone is not life-threatening but predisposes the cattle to secondary bacterial infections, by *Mannheimia haemolytica* for example, which causes bovine pneumonic pasteurellosis (or shipping fever) and can result in death. Understanding the precise molecular mechanisms of these infections is critical so that effective strategies to prevent and treat the diseases can be developed. Mice, guinea pigs and rabbits are commonly used to study bovine respiratory pathogens, however, variability in the susceptibility of different strains of mice to *M. haemolytica* and difficulties translating results across species limit the utility of these approaches.

Using abattoir material, Dr Tucker's team has established a physiologically relevant, rapid, and sensitive *in vitro* respiratory tract organ culture model[2] to analyse host-pathogen interactions following inoculation of *M. haemolytica* and BHV-1. This model has replaced the use of animals in some studies of respiratory disease and has the potential to be used in developing new vaccines. It has also provided the basis for a comparable *ex vivo* pig model[3]. Dr Tucker is now discussing uptake of the bovine model with one of the UK's largest animal health companies.

Replacement *in vitro* assays for quantifiying Clostridial vaccine antigens

NC3Rs funding has led to new *in vitro* assays for use in the development of Clostridial vaccines that have the potential to replace the current animal tests used for regulatory purposes.

Clostridial bacteria produce toxins that cause a wide range of diseases in animals, including tetanus and haemorrhagic enteritis. Vaccines against these bacteria are amongst the most common veterinary treatments. The vaccines are often made from an inactivated form of the toxin, referred to as a toxoid, which can induce an immune response without causing disease. To ensure consistent vaccine quality, it is a regulatory requirement than the amount of toxin and toxoid are measured. Currently this uses tens of thousands of mice in Europe alone, with most tests using lethality as an endpoint.

Dr Keith Redhead and his colleagues at Intervet were funded in 2005 and 2008 to develop *in vitro* assays to quantify Clostridial toxins and toxoids. Five cell line assays have now been identified with suitable sensitivities for various Clostridial species. During 2009, validation of the *Clostridium septicum* assays and their correlation with *in vivo* test data was completed; this work will be submitted to the European regulatory authorities in 2010.

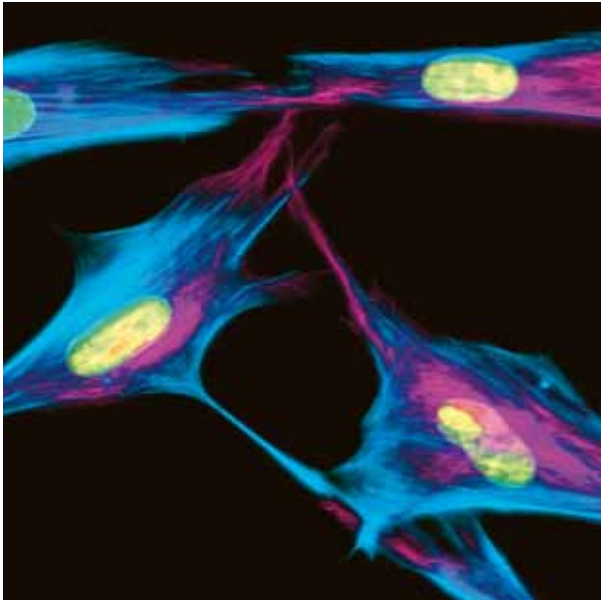
2. Niesalla HS, Dale A, Slater JD, Scholes SFE, Archer J, Maskell DJ & Tucker AW (2009) Critical assessment of an *in vitro* bovine respiratory organ culture system: A model of bovine herpesvirus-1 infection. *Journal of Virological Methods* **158** (1-2), 123-129

3. Nunes SF, Murcia PR, Tiley LS, Brown IH, Tucker AW, Maskell DJ & Wood JLN (2009) An *ex vivo* swine tracheal organ culture for the study of influenza infection. *Influenza and Other Respiratory Viruses* **4**, 7-15.

EXPLOITING SCIENCE AND TECHNOLOGY

Exploiting developments in basic research can advance the 3Rs. Building on the Centre's existing programme of activities with the UK's tissue engineering community, the focus in 2009 was to maximise collaboration between academia and industry. The NC3Rs also launched an initiative on novel approaches to chemical risk assessment.

Dr William Hope (right) and Dr Jayesh Majithiya



Seven new industry/academia collaborations in tissue engineering have formed as a result of the NC3Rs 'speed-networking' event.

The potential of tissue engineering to replace animal use has been demonstrated by the acceptance in 2009 of human epidermis models for skin irritation testing within the European Union. Working closely with the BBSRC and tissue engineers, the NC3Rs has delivered a programme of work to inspire the exploitation of other models – for example for the liver, kidney and lung – by providing opportunities for research funding, communication and collaboration. The success of this programme was highlighted in two reviews published in *Regenerative Medicine*[4] and *Organogenesis*[5] in 2009.

The programme was further progressed in 2009 with a 1.5 day symposium, attended by more than 140 scientists from a wide range of disciplines in industry and academia, and the Centre's first 'speed networking' event. The symposium focused on three main themes; commercial applications, disease models, and stem cells. Presentations covered diverse topics from models of osteoarthritis and sepsis-induced renal failure to the use of human iPS cells to study Huntington's Disease. The keynote lecture was given by Dame Julia Polak from Imperial College London.

The 'speed networking' event provided a unique forum for tissue engineers to showcase their models in '5 minute pitches' to potential industry end-users. The event was attended by over 30 scientists from academia, small biotechnology companies and the chemical and pharmaceutical industries. Seven new industry/academia collaborations have formed as a result of the event. For example, *in vitro* skin alternatives developed by tissue engineers are now being explored by the agribusiness Syngenta as high-throughput screens to assess the effects of chemicals on a range of endpoints without using animals. Information exchanged between delegates has also enabled the tissue engineers to understand how to modify their models in line with industry needs, paving the way for industry to incorporate these models in their research.

The Centre will continue to foster communication and collaboration between tissue engineers and *in vivo* scientists in 2010. Working with smaller groups, the NC3Rs will identify limitations associated with specific disease models and safety assessment endpoints, which might be addressed using tissue engineering, and to inspire research funding proposals in this area.

New approaches to safety testing

The European regulatory environment for chemicals, including REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) and the 7th Amendment to the Cosmetics Directive are driving the development of non-animal methods. Current test methods using animals have not changed significantly over the last few decades and their utility remains controversial. Various reports (for example, from the US National Research Council) have highlighted the limitations of *in vivo* toxicology and the need for mechanism-based assays for hazard identification which are more predictive of human biology and exposure and allow more rapid screening of chemicals than is currently possible.

In November 2009, the NC3Rs hosted a 1.5 day workshop to explore aligning the latest developments in science and technology with chemical risk assessment. Focusing on replacing the use of animals for repeat dose toxicity testing the workshop was attended by 30 experts from disciplines not normally associated with toxicology, including mathematical modelling, molecular imaging and epigenetics. The delegates were challenged to brainstorm a hypothetical testing paradigm and then to pitch it to an expert panel in a '*Dragon's Den*' format. The novel ideas generated will be used as the basis for the NC3Rs future activities in this area, including informing future research priorities.

4. Holmes A, Brown R & Shakesheff K (2009) Engineering tissue alternatives to animals: applying tissue engineering to basic research and safety testing. *Regenerative Medicine* **4** (4), 579-592
5. Westmoreland C & Holmes A (2009) Assuring consumer safety without animals: applications for tissue engineering. *Organogenesis* **5** (2), 67-72

CHANGING THE REGULATORY ENVIRONMENT

International regulatory guidelines on the safety assessment of chemicals and pharmaceuticals have an impact on animal use worldwide. During 2009, the NC3Rs continued to work with industry and regulators to implement the 3Rs within the existing regulatory framework and by facilitating regulatory change.

The NC3Rs is generating scientific evidence to support European and international acceptance of the Fixed Concentration Procedure, to reduce and refine animal use for acute inhalation toxicity testing.

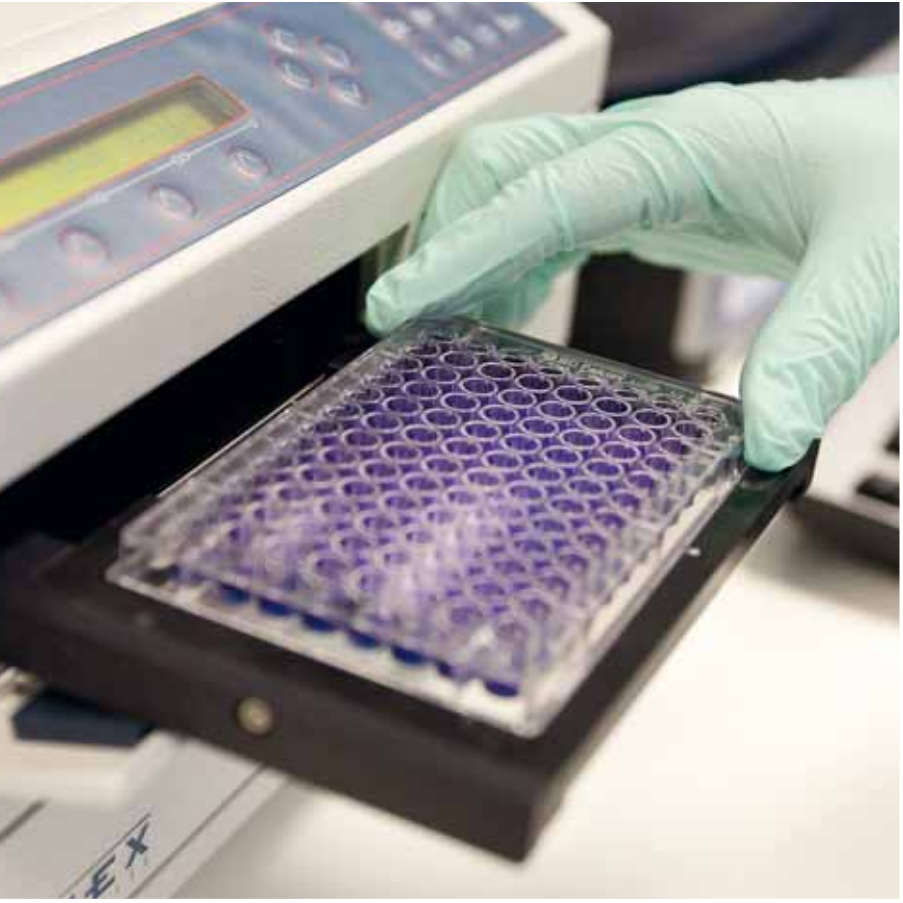
Reduction and refinement in acute inhalation toxicity testing

Information on acute oral toxicity is one of the base set of requirements for all chemicals falling under the remit of the REACH regulation. For chemicals produced in volumes greater than ten tonnes per annum, acute toxicity testing by a second route of exposure is also required, and for many chemicals this will be the inhalation route. This test is also commonly required for chemicals that fall under other regulations (e.g. pesticides). Acute toxicity tests are of particular concern for animal welfare as they generally require death or impending death as the endpoint.

In collaboration with the Health Protection Agency, the Health and Safety Executive, and the chemical industry, the NC3Rs is leading a project to provide an evidence base to support European Union and international acceptance of the Fixed Concentration Procedure (FCP) – an alternative method for assessing acute inhalation toxicity. The FCP uses less than half the animals of current test methods and, moreover, provides a refinement because it does not require severe toxicity or death of the animal as an endpoint. Instead, 'evident' toxicity – signs of toxicity predicting that animals exposed to a higher level of the test chemical would experience severe toxicity or die – is employed.

The NC3Rs has commissioned Dr Nigel Stallard and Dr Charlotte Price from the University of Warwick to perform a statistical comparison of the performance of the FCP with the test methods currently used, as well as the potential for sex differences in response to exposure by the inhalation route – this is necessary because the FCP proposes testing animals of just one sex. Two papers reporting these findings are currently undergoing peer review.

A potential barrier to acceptance of the FCP is its reliance on evident toxicity as an endpoint, with some regulatory bodies reluctant to move away from using death. To address this, the Centre held an expert meeting in June 2009 involving inhalation toxicology experts, animal technicians and representatives from the European Centre for the Validation of Alternative Methods and the US Interagency Coordinating Committee on the Validation of Alternative Methods, to discuss how evident toxicity can be reliably identified and used. Based on these discussions, the NC3Rs is currently leading the development of guidelines on using clinical signs of evident toxicity. The results of this work will be presented to the Organisation for Economic Cooperation and Development in 2010 as a first step to achieving international acceptance of this alternative test method.



Challenging the requirement for acute toxicity testing of pharmaceuticals and chemicals

The requirement for single dose acute toxicity testing of pharmaceuticals in rodents prior to first time in man studies was removed from the international regulatory guidelines (ICH M3) in 2009. This was as a direct result of work led by the NC3Rs and AstraZeneca, and involving 17 European pharmaceutical companies and contract research organisations, which had previously demonstrated that the test was no longer required. This is especially important as it removes the only test in pharmaceutical pre-clinical development where the death of the animal is an endpoint. Building on its capability in this area, the Centre has now focused on challenging the requirement for acute toxicity data to support pharmaceutical overdose in man, and minimising the use of this test in chemical risk assessment.

Despite the revision of ICH M3, acute toxicity studies may still be required in the drug development process to assess overdose in man prior to the drug becoming widely available. To assess whether acute toxicity data is actually used to determine the effect of overdose, the NC3Rs/industry partnership carried out a pilot survey of international Poison Centres in 2009[6]. Initial data from one US and nine European Poison Centres suggest that most do not use acute toxicity data from animals to determine the effects of overdose in man, and may get more useful information from other animal studies (e.g. those that assess target organ toxicity) which are carried out as part of the drug development process. To build on the survey, the NC3Rs has organised a workshop for January 2010 with representatives from Poison Centres, regulatory bodies and the pharmaceutical and chemical industries to consider whether acute toxicity data continues to be required.

The success of its work on acute toxicity testing in the pharmaceuticals sector has led the NC3Rs to review acute toxicity testing in the chemical sector. The regulatory framework for the safety assessment of chemicals is complex, and requirements vary from sector to sector (e.g. pesticides, industrial chemicals, and consumer products) and from region to region. Inconsistent application of protocol requirements and alternative approaches across sectors and regions can contribute to unnecessary and redundant animal testing. The Centre established an expert working group including representatives from industry and regulatory bodies to identify opportunities to waive acute toxicity tests, or to use alternative approaches that can replace, reduce or refine *in vivo* testing by identifying areas of redundant testing (i.e. where there is duplication of testing, or where testing can be avoided through the use of alternative means of generating the required data). The review, which has been accepted for publication by *Critical Reviews in Toxicology*[7], focuses on testing for six key acute toxicity endpoints that are

6. Robinson S & Chapman K (2009) Are acute toxicity studies required to support overdose for medicines? *Regulatory Toxicology and Pharmacology* **55** (1), 110
7. Creton S, Dewhurst IC, Earl LK, Gehen SC, Guest R, Hotchkiss JA, Indans I, Woolhiser M & Billington R (in press) Acute toxicity testing of chemicals: opportunities to avoid redundant testing and use alternative approaches. *Critical Reviews in Toxicology*

Revision of ICH M3 regulatory guidelines has been directly influenced by the NC3Rs, ending the requirement for single dose acute toxicity testing in rodents for pharmaceuticals to support first time in man studies.

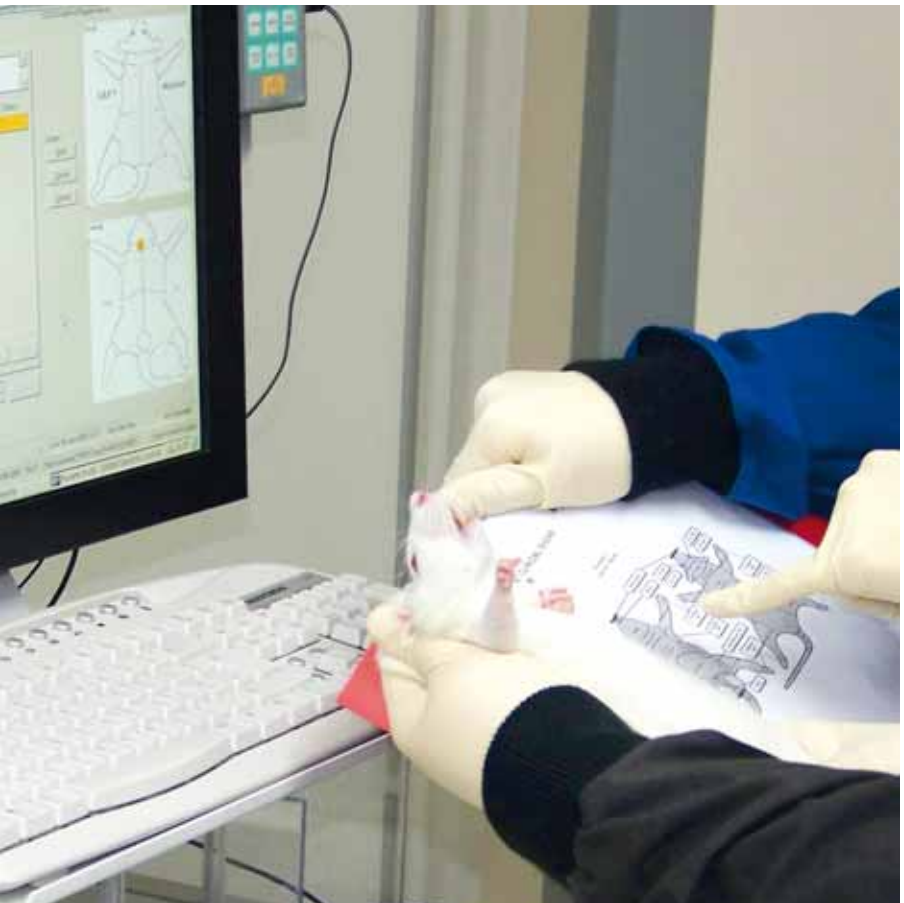
commonly required by regulatory authorities (acute oral, dermal and inhalation toxicity, skin and eye irritation, and skin sensitisation) and highlights a number of areas where alternative approaches or waiving of testing can reduce and refine animal use.

The paper includes historical data analyses demonstrating that acute dermal testing of pesticides rarely provides additional information of value when testing by the oral route has already been conducted. This complements work being undertaken by a task force of the European Partnership on Alternative Approaches to Animal Testing (EPAA). To promote future regulatory change, the NC3Rs is currently collaborating with the EPAA to organise a workshop in 2010 to share these findings with international regulators.

Guidance on dose selection

Failure to select the appropriate dose for toxicity studies can lead to unnecessary morbidity and mortality, and the requirement to repeat studies. In 2009, the NC3Rs and LASA published guidelines on dose selection in toxicology studies carried out for regulatory purposes[8]. The guidelines were developed with toxicologists from the major pharmaceutical companies and contract research organisations in the UK and the Animals (Scientific Procedures) Inspectorate.

Aimed at new study directors, the guidance sets out recommendations, with worked examples, for selecting dose levels for general toxicity tests, including preliminary dose range finding studies. It is endorsed by the ABPI and British Toxicology Society.



8. Robinson S, Chapman K, Hudson S, Sparrow S, Spencer-Briggs D, Danks H, Rose H, Everett D, Mulier B, Old S & Bruce C (2009) *Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals*. LASA, Tamworth, and NC3Rs, London.

The NC3Rs has identified opportunities to halve the number of primates used per mAb in development.

Minimising primate use in monoclonal antibody development

The use of non-human primates (NHPs) in scientific procedures was high on the political agenda in 2009, following publication of the European Commission's proposal to revise Directive 86/609/EEC and an opinion from its Scientific Committee on Health and Environmental Risks on *'The need for non-human primates in biomedical research, production and testing of products and devices'*. The latter adds to the other reports in this area, all of which have supported greater efforts on the 3Rs. The NC3Rs has continued to take the lead in this area in 2009, working with the ABPI and international pharmaceutical and biotechnology companies on three areas; monoclonal antibodies (mAbs), pharmacokinetics and abuse potential studies. The main priorities have been disseminating the output from the industry data sharing that the NC3Rs has coordinated and inputting into the addendum to the ICH S6 guidelines.

One of the main factors driving a rise in NHP use worldwide is the increasing development of mAbs as therapies for human diseases such as cancer and immune-related conditions.

Approximately a third of the molecules in the drug pipeline are biologics and it is predicted that within three years the number of biologics could outstrip the number of new chemical entities in development. The high target and species specificity of many mAbs means that often the only relevant species for safety and toxicity testing is the NHP, usually the cynomolgus monkey.

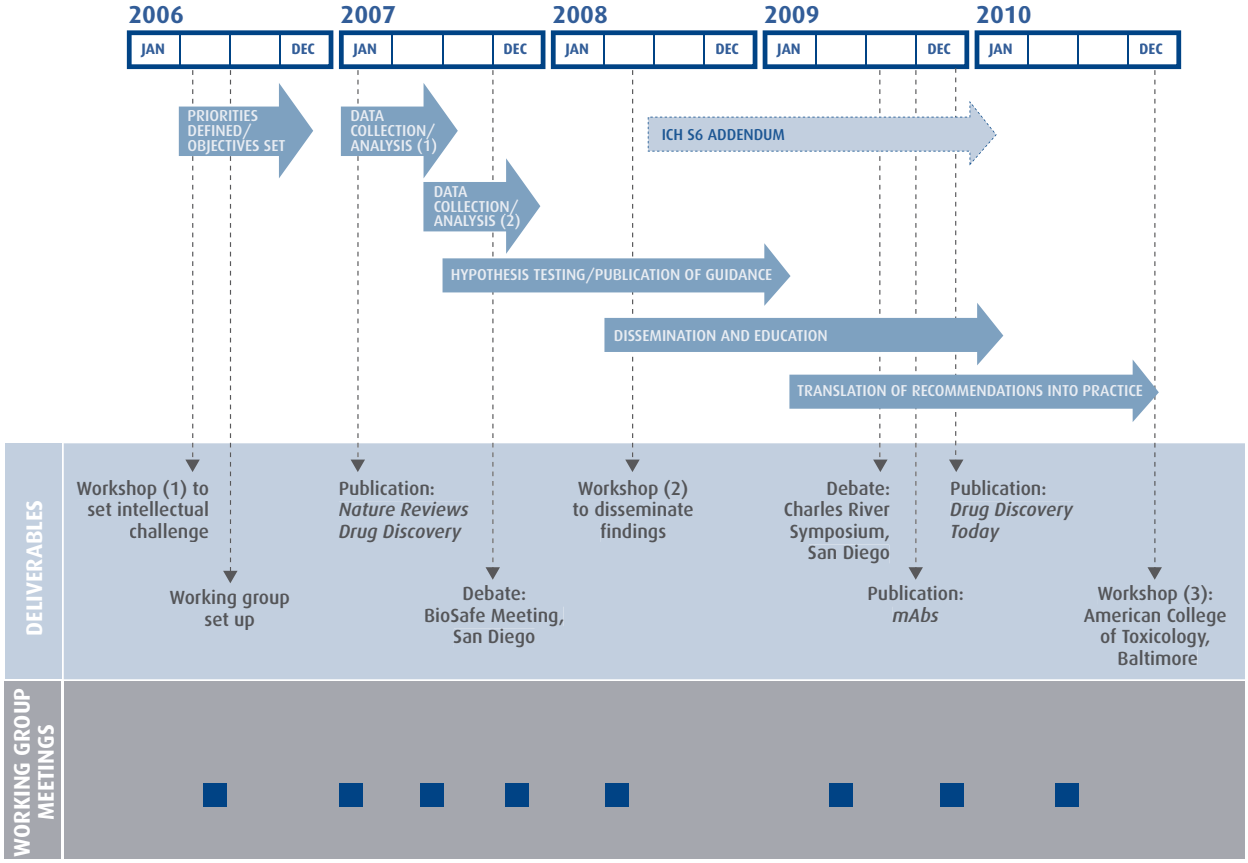
Working with 24 international pharmaceutical and biotechnology companies and regulatory authorities, the NC3Rs has previously acted as an honest broker for sharing data on 120 unique mAbs, including information on pre-clinical study designs, mAb potency and the use of homologous proteins in rodents. Based on the data collected and the biology of mAbs, opportunities have been identified to up to halve the number of NHPs used to 52 animals per mAb, by decreasing the number of dose groups, recovery animals used and chronic studies performed. These opportunities were shared with a wider audience from industry, academia and regulatory bodies through publications in *mAbs*[9] and *Drug Discovery Today*[10].

9. Chapman K, Pullen N, Coney L, Dempster M, Andrews L, Bajramovic J, Baldrick P, Buckley L, Jacobs A, Hale G, Green C, Ragan I & Robinson V (2009) Preclinical development of mAbs: considerations for the use of NHPs. *mAbs* **1** (5), 500-511
10. Chapman K, Pullen N, Andrews L & Ragan I. The future of non-human primate use in mAb development. *Drug Discovery Today* (in press)

The NC3Rs also works closely with other expert groups and committees from the US and elsewhere in Europe who are actively identifying opportunities to improve the pre-clinical development of mAbs, for example the Biosafe Committee and the PhRMA Biopharmaceuticals Technical Group. Many of these initiatives also have implications for minimising NHP use.

2009 also marked the publication of the draft addendum to the ICH S6 regulatory guidelines on *'Pre-clinical safety evaluation of biotechnology-derived pharmaceuticals'*. The addendum, which is expected to be finalised in 2010, includes the potential for the opportunities identified by the NC3Rs/industry collaboration to be implemented, with the prospect of global impact.

Minimising NHP use in mAb development – timeline



This illustrates the timeline for the NC3Rs/industry initiative to minimise NHP use in mAb development. Data collection and analysis is coordinated by the NC3Rs and expert input is provided through working group meetings. The impact of the initiative has been achieved through international workshops, publications in key journals in the field, and participation in major scientific meetings.

RAISING AWARENESS OF THE 3RS

The NC3Rs raises awareness across the biosciences sector of opportunities to implement the 3Rs, and promotes greater involvement in the 3Rs agenda, through its regular events, comprehensive website, publications and annual 3Rs prize.

Dr Ian Ragan

More than 850 delegates attended the 11 events organised by the NC3Rs in 2009.

Events

The NC3Rs has a reputation for organising high-quality scientific symposia and workshops in a broad range of research areas. These events provide unique forums for discussion and interaction on the 3Rs and have become an important part of the biosciences calendar. More than 850 delegates attended the eleven events organised by the Centre in 2009 (see next page).

A particular highlight was the Centre's annual Primate Welfare Meeting which continues to play an important and unique role in bringing together the primate using community to network and share information on contemporary best practice in accommodation, care and use. The meeting, which was organised jointly with European Primate Veterinarians, focused on refining techniques for scientific procedures. Presentations were given on topics such as magnetic resonance imaging, anaesthesia and analgesia and post-operative care, disease models, humane endpoints, and blood pressure and electrocardiogram measurements. One hundred and forty delegates from more than 60 organisations and 15 countries attended, demonstrating the international outreach of the NC3Rs.

Website

The Centre's website (**www.nc3rs.org.uk**) is a comprehensive online 3Rs resource and the main portal for information on the work of the NC3Rs. In 2009, the site received nearly 300,000 page views (a 51% increase on the previous year) and significant new functionality was added. This included social book-marking to facilitate information transfer, and RSS news feeds to keep users up-to-date with new content. The NC3Rs also highlights new content by distributing an e-newsletter; this has almost 1,200 individual subscribers and is circulated widely through discussion groups and other electronic networks.

CENTRE PUBLICATIONS FOR 2009

Chapman K, Pullen N, Coney L, Dempster M, Andrews L, Bajramovic J, Baldrick P, Buckley L, Jacobs A, Hale G, Green C, Ragan I & Robinson V. Preclinical development of monoclonal antibodies: considerations for the use of non-human primates. *mAbs* **1** (5), 500-511

Creton S, Billington R, Davies W, Dent MP, Hawksworth GM, Parry S & Travis KZ. Application of toxicokinetics to improve chemical risk assessment: implications for the use of animals. *Regulatory Toxicology and Pharmacology* **55** (3), 291-299

Holmes A, Brown R & Shakesheff K. Engineering tissue alternatives to animals: applying tissue engineering to basic research and safety testing. *Regenerative Medicine* **4** (4), 579-592

Holmes AM, Rudd JA, Tattersall FD, Aziz Q & Andrews PL. Opportunities for the replacement of animals in the study of nausea and vomiting. *British Journal of Pharmacology* **157** (6), 865-880

Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, Hutton J & Altman DG. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS ONE*, **http://dx.plos.org/10.1371/journal.pone.0007824**

Lave T, Chapman K, Goldsmith P & Rowland M. Human clearance prediction: shifting the paradigm. *Expert Opinion on Drug Metabolism and Toxicology* **5** (9), 1039-1048

Robinson S & Chapman K. Are acute toxicity studies required to support overdose for new medicines? *Regulatory Toxicology and Pharmacology* **55** (1), 110

Robinson V. Less is more: reducing the reliance on animal models for nausea and vomiting research. *British Journal of Pharmacology* **157** (6), 863-864

Robinson V. Alternatives to animals. *New Scientist* **201** (2698), 22

Robinson V. Leading the search for alternatives. *Science in Parliament* **66** (3), 16-17

Watson T. 2009: A landmark year for the 3Rs. *Lab Animal Europe* **9** (12), 3

Westmoreland C & Holmes A. Assuring consumer safety without animals: applications for tissue engineering. *Organogenesis* **5** (2), 67-72

In Press

Chapman K, Pullen N, Andrews L & Ragan I. The future of non-human primate use in mAb development. *Drug Discovery Today*

Creton S, Dewhurst IC, Earl LK, Gehen SC, Guest R, Hotchkiss JA, Indans I, Woolhiser M & Billington R. Acute toxicity of chemicals: opportunities to avoid redundant testing and use alternative approaches. *Critical Reviews in Toxicology*

Holmes AM, Creton S & Chapman K. Working in partnership to advance the 3Rs in toxicity testing. *Toxicology*

Robinson V. The Importance of anniversaries. *Alternatives to Laboratory Animals*


Phil Willis MP

EVENTS IN 2009

Stakeholder Meeting
28 January, London

Annual event providing a scientific review of the Centre's progress and future plans.

The 3Rs today
25 March, London

Poster event to showcase UK 3Rs activity to parliamentarians.

Tissue engineering: a new dimension to animal replacement – joint with BBSRC
1 – 2 April, London

Symposium to highlight the replacement potential of tissue engineering and stem cell technologies.

Encouraging commercial uptake of tissue engineering: a speed-networking event
1 April, London

Half-day event providing tissue engineers with an opportunity to 'pitch' their models to chemical and pharmaceutical companies.

New opportunities for the 3Rs – joint with Biosciences Federation
23 April, London

Annual symposium to highlight scientific and technical advances in the 3Rs.

Grant Holders' Meeting
21 May, London

Biennial meeting to bring together NC3Rs grant holders for networking and information exchange.

Beyond animal research
4 June, Cheltenham

Public event at the Cheltenham Science Festival to announce the winner of the NC3Rs/*New Scientist* essay competition.

Animal Technicians' Symposium
23 September, London

Annual symposium for animal technicians, sponsored by AstraZeneca, focusing on animal welfare and refinement issues.

Primate Welfare Meeting – joint with European Primate Veterinarians
28 October, London

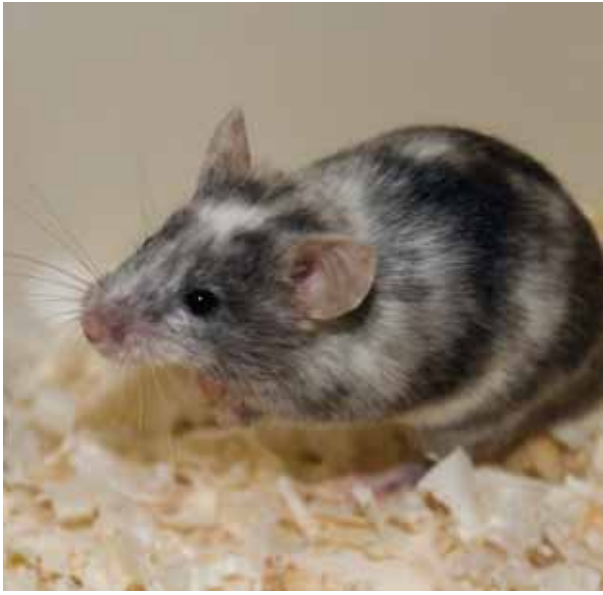
Annual event bringing together researchers, veterinarians and animal care staff with a common interest in the welfare of laboratory-housed NHPs.

Novel approaches to safety assessment
11 – 12 November, London

Workshop to explore how recent advances in science and technology in a broad range of disciplines could be used to develop new approaches to chemical risk assessment.

Animal models of asthma: value, limitations and opportunities for alternative approaches
23 November, London

Workshop to explore the development of better models of asthma with improved scientific and clinical relevance and reduced reliance on the use of animals.



The 3Rs prize winner's method for deriving embryonic stem cells has the potential to dramatically reduce the number of mice used in experiments worldwide.

Publications

2009 was a productive year for publications co-authored by the NC3Rs scientific staff and renowned experts from a variety of fields. Sixteen research papers and editorials, reporting on Centre-led activities, were published (see page 21). A laboratory poster and information leaflet were also launched to demonstrate the benefits of the 3Rs and improve knowledge of the Centre's key activities among a wider audience at research establishments throughout the UK.

The NC3Rs also worked with animal welfare organisations on two reports published in 2009. The first was a report published in *Laboratory Animals*[11] on refining primate husbandry practices and scientific procedures. Co-authored and edited by NC3Rs staff, the report was widely distributed by the Centre and received 1,500 website downloads in its first month of publication. The NC3Rs also participated in an initiative, led by the RSPCA, which produced guidance on archiving frozen (cryopreserved) embryos or gametes from GM mice[12] as a means of reducing and refining their use.

3Rs Prize

The Centre's annual 3Rs Prize, sponsored by GSK, rewards excellent published research that has significant 3Rs and scientific impacts. The prize of £10k is awarded for a research paper published in the last two years. The 2009 winner is Dr Jennifer Nichols, from the University of Cambridge, who has developed an optimised culture medium for growing mouse embryonic stem (ES) cells and utilised this medium to derive ES cells from non-obese diabetic (NOD) mice for the first time[13]. The work published in *Nature Medicine* will reduce the number of mice used in investigations of type 1 diabetes and also has the potential to further reduce animal use for other disease models.

ES cells are derived from early embryos and can be grown indefinitely in culture. They are a powerful tool in biomedical research because of their 'pluripotency', which means they can be transformed into all other cell types in the body, making them widely used in *in vitro* experiments and to generate disease models in mice. Previously, understanding which genes play a role in type 1 diabetes involved breeding NOD mice with strains which had the gene of interest modified. This lengthy process required at least ten generations of breeding, involving many hundreds of animals, before the mice had a suitable genetic background for conducting the experiment.

The derivation of ES cells from the NOD mouse allows its genes to be directly manipulated to study type 1 diabetes without many generation of backcrossing, dramatically reducing the number of mice required per experiment. The NOD ES cells are now freely available to the research community, potentially reducing the number of mice used in type 1 diabetes research worldwide.

The new medium, which contains a novel mixture of cell growth factors and inhibitors but no animal products, has also allowed the derivation of ES cells from every strain of mouse tested so far with extremely high efficiency. Dr Nichols' laboratory has made this technology available so that it can also be applied to other mouse models of disease where deriving ES cells has previously proven impossible. The prize will be used to host researchers from other groups, either individually or in workshops, so they can learn the culture technique to ensure it is quickly and widely disseminated to other laboratories.

11. Joint Working Group on Refinement (2009) Refinements in husbandry, care and common procedures for non-human primates. Ninth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (M Jennings & MJ Prescott, eds.) Laboratory Animals **43** (S1), 1-47
12. BBSRC, Cancer Research UK, MRC, NC3Rs & RSPCA (2009) Sharing and archiving of genetically altered mice: opportunities for reduction and refinement. RSPCA: Horsham, West Sussex.
13. Nichols J et al. (2009) Validated germline competent embryonic stem cell lines from non-obese diabetic mice. *Nature Medicine* **15** (7), 814-818

APPLYING THE 3RS IN PUBLICLY-FUNDED RESEARCH

Over half of the scientific procedures performed on animals are carried out in universities and other public institutions, making working with the research funding bodies to embed the 3Rs and improve standards in animal research an important role for the NC3Rs. During 2009, the Centre expanded this role, including highlighting the need for better reporting of animal studies.

The NC3Rs has carried out the largest survey on the quality of experimental design and reporting of animal research.

Working with major biosciences research funders

The NC3Rs continues to provide the UK's research councils and charitable funding bodies with the expertise to ensure the 3Rs are implemented in the research that they support. The Centre reviews all grant, fellowship and studentship applications to the MRC, BBSRC and Wellcome Trust involving the use of NHPs, cats, dogs or equines. Its involvement in the peer review process helps to identify and address any animal welfare issues, to ensure that any 3Rs opportunities are exploited, and to monitor the implementation of guidelines, such as *'Responsibility in the Use of Animals in Bioscience Research'*, produced with the funders to support best practice.

During 2009, the NC3Rs identified a range of opportunities for avoiding animal use, improving experimental design and introducing refinements. Over 50 applications were reviewed, as well as eight quinquennial review reports from MRC research units, centres and institutes. For the first time, the Wellcome Trust Translation Awards, aimed at commercialising new technologies to address unmet needs in healthcare, were included in this process. The Centre also agreed to review fellowship proposals involving higher species submitted to the Wellcome Trust/DBT (Department of Biotechnology) India Alliance – an independent charitable trust established to support current and future leaders of Indian biomedical science.

Experimental design and reporting

The NC3Rs has conducted the largest and most comprehensive survey to-date of the quality of experimental design, reporting and statistical analysis of published research using animals.

The survey, co-funded by the National Institutes of Health Office of Laboratory Animal Welfare, assessed 271 randomly selected papers reporting experiments using mice, rats or NHPs conducted in publicly-funded institutions in the USA and UK. The results, published in 2009 by *PLoS ONE*[14], showed that many of the papers assessed lacked important information; for example, only 59% reported the objectives of the study, the number of animals used, and essential details about the animals such as sex, age or weight, and strain. Of the studies that carried out a statistical analysis, only 70% reported the statistical methods used and presented the results with a measure of variation.

Improving experimental design and reporting is important to ensure that the minimum number of animals is used to achieve scientific objectives, and that the knowledge gained from each animal and experiment is maximised. The Centre has used the survey results as a basis to develop guidelines to improve the quality of reporting of animal research. To optimise their relevance and utility, the guidelines have been developed in consultation with researchers across a range of scientific disciplines, journal editors, and representatives of the major research funding bodies. The guidelines set out the information required to enable suitably qualified readers to critically assess papers reporting animal research, and will be published in 2010. Over the next 12 months, the NC3Rs will begin to develop a range of resources to help researchers ensure that their experiments are appropriately designed.

14. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, Hutton J & Altman DG (2009) Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS ONE* **http://dx.plos.org/10.1371/journal.pone.0007824**

LOOKING AHEAD TO 2010

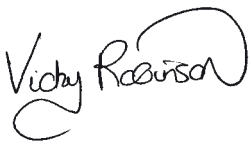
3Rs research has gained a new legitimacy as a desirable scientific goal. The NC3Rs has been at the heart of the revolution to modernise the 3Rs, funding leading scientists and groups across a range of disciplines.

Supporting research through open competition has been a successful funding model for delivering 3Rs solutions; however as the NC3Rs evolves it is important to build on the experience gained to date and to consider new opportunities for enhancing the Centre's research investment. With this in mind, 2009 saw the launch of the NC3Rs PhD Studentship Scheme which, by influencing the training and development of future scientists, will lead to a fundamental shift in the perception of the 3Rs.

Over the next 12 months, we will introduce other new funding mechanisms, including for pilot and proof of concept projects, which will operate in addition to the Centre's support for innovation through response mode funding. Going forwards, we will also require greater flexibility to define and shape our research portfolio in order to better support the priorities identified through the Centre-led programmes. This is illustrated by the work we are leading on using the 3Rs to provide new models and tools for key areas of health research. Asthma has been selected as the first area and, following a joint NC3Rs/MRC workshop in November 2009 with the UK's leading respiratory disease biologists, a list of research priorities have been identified.

Funding research also generates valuable new material for us and our grant holders to use in public engagement activities. The main theme of our grant holder meeting in May was how we could assist those we fund in working with the media or giving talks in schools for example. Indeed, the 50th anniversary of the 3Rs provided a platform for us to take a more active role in public engagement activities and the plan is to build on this over the next few years - improving public awareness on the 3Rs and shifting the debate on animal research away from the polarised extremes. The 3Rs terminology is not instinctively accessible and a first step will be to consider how to develop effective language and messages for a public audience. For the first time in 2009 the Ipsos MORI poll on public attitudes to animal research, commissioned annually by the Government, included questions directly relevant to the NC3Rs. The results, available later this year, will provide a useful benchmark for our activities.

As we expand our public engagement on the 3Rs, it will be important to work closely with our scientific partners to ensure this is done effectively and reaches the widest possible audience. We will seek input on our plans during 2010.



Dr Vicky Robinson, Chief Executive

FINANCIAL SUMMARY

This annual report describes the activities of the NC3Rs for the calendar year 2009. The NC3Rs accounting period runs from 1 April to 31 March each year. The information provided below covers the period 1 April 2008 to 31 March 2009.

For the year ending 31 March 2009

The total income for this financial period was £4,529,498; 57% higher than the period April 2007 to March 2008. This largely reflects additional funding from the MRC and BBSRC.

The annual budget is agreed by the NC3Rs Board. Total expenditure increased from £2,171,709 in 2007/08 to £3,188,414 in 2008/09. This can primarily be accounted for by increased grant and operating costs.

Board costs include travel for members to meetings and associated honorariums. In the period April 2008 to March 2009, Board costs were £12,720; 50% lower than in the previous financial year. The previous year's costs were higher as a result of recruitment costs (e.g. advertising) for the new NC3Rs Board chairman.

Categorisation of expenditure for programme and operating costs has been revised to ensure greater transparency. Programme costs cover initiatives led by the NC3Rs Office. This includes costs for workshops, symposia, working groups and the salaries of scientific staff who lead these initiatives. In the period April 2008 to March 2009, expenditure on programme costs was £906,312; 27% higher than in the previous financial year. This reflects the cost of ongoing and new initiatives.

Operating costs include salaries for core administrative staff, travel expenses and training, recruitment, stationery and publishing costs. In the period April 2008 to March 2009, expenditure on operating costs was £343,255; 129% higher than in the previous financial year. This was due to rental and service charges which in previous years had been a notional charge and had been refunded by the MRC.

Grant expenditure was £1,926,127 in the period April 2008 to March 2009; 50% higher than in the previous financial year. This reflects the ongoing expenditure of grants awarded in 2004, 2005, 2006, 2007 and 2008. Note that grants are awarded for a period of up to three years; however, they can start up to six months after the award date, which can result in grant spending falling in a later financial year.

Income	**2008/09**	**2007/08**
Government	£3,818,000	£2,630,000
Charity	£580,869	£62,904
Industry	£130,629	£199,000
Total	**£4,529,498**	**£2,891,904**

Expenditure	**2008/09**	**2007/08**
Board costs	£12,720	£25,388
Programme costs	£906,312	£715,862
Operating costs	£343,255	£150,036
Grant costs (includes Small Awards)	£1,926,127	£1,280,423
Total	**£3,188,414**	**£2,171,709**

Research funding expenditure

Financial year	Total awarded	Total spent
2004/05	£519,157	£118,379
2005/06	£988,436	£268,990
2006/07	£1,467,222	£815,653
2007/08	£2,467,711	£1,280,423
2008/09	£2,646,080	£1,926,127

BOARD, PANEL MEMBERS, AND STAFF

Board

Professor Ian Kimber (Chair)
University of Manchester

Dr Julia Fentem (Deputy Chair)
Unilever

Dr Phil Botham
Syngenta *(from July 2009)*

Professor Jamie Davies
University of Edinburgh
(from July 2009)

Dr Lesley Heppell
BBSRC

Professor Jane Hurst
University of Liverpool

Dr Maggy Jennings
RSPCA

Dr Tony Peatfield
MRC

Dr Vicky Robinson
NC3Rs

Dr Malcolm Skingle CBE
GlaxoSmithKline
(from October 2009)

Mr Neil Yates
University of Nottingham
(from July 2009)

Grant assessment panel for 2009

Professor Sir Andrew McMichael (Chair)
University of Oxford

Professor Jane Hurst (Deputy Chair)
University of Liverpool

Professor Innes Cuthill
University of Bristol

Professor Andrew Derrington
University of Kent

Dr Colin Dunn
Charles River

Professor Paul Flecknell
Newcastle University

Dr Jeffrey Fry
University of Nottingham

Dr Tim Hammond
AstraZeneca

Professor Ian Kimber
University of Manchester

Professor Sheila MacNeil
University of Sheffield

Mr Terry Priest
University of Manchester

Dr Vicky Robinson
NC3Rs

Dr Carl Westmoreland
Unilever

Co-opted members for 2009:

Dr Lee Buttery
University of Nottingham

Professor Lindy Holden-Dye
University of Southampton

Professor Ian Jackson
MRC Human Genetics Unit

Dr Cahir O'Kane
University of Cambridge

Professor Rodney Phillips
University of Oxford

Studentship assessment panel for 2009

Dr Malcolm Skingle CBE (Chair)
GlaxoSmithKline

Professor Paul Bolam
University of Oxford

Professor Bill Dawson
Bionet Ltd

Professor Christine Nicol
University of Bristol

Dr Sally Robinson
AstraZeneca

Dr David Tattersall
Pfizer Ltd

Professor Dominic Wells
Imperial College London

Co-opted member for 2009:

Professor David Lee
Queen Mary, University of London

3Rs prize selection panel for 2009

Professor Ian Kimber (Chair)
University of Manchester

Professor Paul Flecknell
Newcastle University

Dr Declan Mulkeen
MRC

Dr Ian Ragan
CIR Consulting Limited

Professor Patrick Vallance
GlaxoSmithKline

Professor Paul Wiles
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Dr Harriet Warburton
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Dr Emma Willoughby
Research Funding
(maternity cover)

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ACKNOWLEDGEMENTS

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LASA

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Professor Paul Flecknell
Newcastle University

Dr James Kirkwood OBE
UFAW

Professor Nancy Rothwell DBE
University of Manchester

GLOSSARY

ABPI:	Association of the British Pharmaceutical Industry
ASPA:	Animals (Scientific Procedures) Act 1986
BBSRC:	Biotechnology and Biological Sciences Research Council
BHV-1:	Bovine herpesvirus 1
EDM:	Early Day Motion
FCP:	Fixed Concentration Procedure
GSK:	GlaxoSmithKline
ICH:	International Committee on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LASA:	Laboratory Animal Science Association
MRC:	Medical Research Council
NASA:	National Aeronautics and Space Administration
PhRMA:	Pharmaceutical Research and Manufacturers of America
REACH:	Registration, Evaluation, Authorisation and restriction of CHemicals
RSS:	Really Simple Syndication

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