National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

PhD Studentship Review

Pioneering Better Science

2017

About the NC3Rs

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is a scientific organisation that leads the discovery and application of new technologies and approaches that minimise the use of animals in research and improve animal welfare (the 3Rs).

We collaborate with scientists and organisations from across the life sciences sector, nationally and internationally, including universities, the pharmaceutical, chemical and consumer products industries, other research funders, and regulatory authorities.

We support the commitment of the scientific community to the 3Rs by funding research and early career development, facilitating open innovation and the commercialisation of 3Rs technologies, and stimulating changes in policy, regulations, and practice.

Further information can be found at www.nc3rs.org.uk

Contents

Introduction

The focus of this review is the NC3Rs PhD studentship scheme, which embeds the 3Rs in the training of new generations of graduate scientists.

Supporting scientists at every career stage to engage with the 3Rs is critical for ensuring the scientific and cultural changes necessary to sustain the development of knowledge, technologies and skills that underpin the replacement, reduction and refinement of the use of animals in research (the 3Rs).

Providing 3Rs training for early career researchers has the potential to have a long-term impact on how they design and conduct their experiments, and ultimately, as potential research leaders, how they influence their field and the work of others. For these reasons, we have introduced funding schemes for PhD studentships, and fellowships for postdoctoral scientists with varying levels of experience.

The PhD studentship scheme was launched in 2009. Since then we have funded 87 studentships, a total value of £8.25 million. Our commitment goes beyond providing funding. We also support the professional growth of the student, including through a Summer School where the student can learn 3Rs-relevant skills, how to critically appraise research models from scientific and animal welfare perspectives, and how to communicate the importance and impact of their work to different audiences.

In this review, we summarise the impact that our studentship scheme has had to date. We provide information on the number of awards by year, institution and scientific category, as well as the breakdown of awards under each ‘R’, and outputs such as publications. We also highlight, through a series of case studies, the scientific and 3Rs impacts achieved. The case studies describe research materials developed during the studentship, additional funding secured by the supervisors and students, dissemination of research outputs, and recognition through prizes and other awards.

The studentship scheme: Portfolio analysis

Our response mode PhD studentship scheme currently provides £90k over three years.*

The awards are made directly to principal investigators through a competitive application process. This allows the NC3Rs to establish close working relationships with grant holders and students, and in turn, this personalised approach drives change, providing the support for grant holders and students to seek out opportunities to maximise the 3Rs impact of their work.

*Awards were initially available as three or four-year awards. This was changed to three-year awards only in 2011.

Studentship awards by year and institution

Table 1 shows the number of awards made by year, as well as the number of applications received. Since 2011, we have committed to funding a minimum of ten studentships per year, although depending on the available budget, in some years we have made additional awards.

Table 1: Studentship awards by year, 2009 to 2016

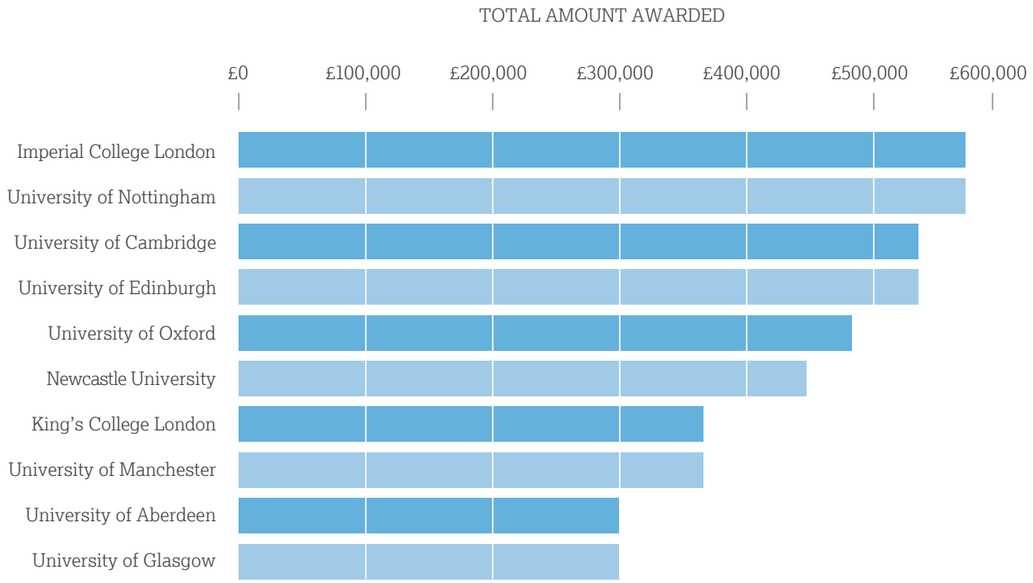
	Number of applications	Number of awards	Success rate*
2016	74	11	15%
2015	136	15	11%
2014	46	10	22%
2013	68	14	21%
2012	53	12	23%
2011	89	15	17%
2010	76	5	7%
2009	48	5	10%

*Rounded up to the nearest whole percent.

A new collaboration was established in 2015 with the British Heart Foundation (BHF) to fund up to three joint studentships per year. These jointly badged studentships are focused on achieving 3Rs impact, and supporting 3Rs training and development in cardiovascular research, where many of the current animal models have limitations for translation to human disease or are associated with animal welfare concerns.

All applications to the studentship scheme, including those that fall within the NC3Rs/BHF remit, are assessed using the same two key criteria: the scientific quality of the work proposed, and its potential 3Rs impact. The quality of the training environment and the track record of the supervisor are also taken into account.

Figure 1: Top ten funded institutions by amount awarded, 2009 to 2016



The number of applications received has fluctuated from 48 to 136 per year and the average success rate for the studentship scheme is 16%. This cannot be compared to the success rates for the block training grants awarded directly to research organisations by the Research Councils; however, it is broadly in line with average success rates for the major UK biomedical charities, which also award their studentships on an individual grant holder basis.

Awards have been made to 37 universities across the UK. Figure 1 shows the top ten funded institutions based on the amount awarded. 47 awards were made to these ten institutions, totalling £4.5 million – 54% of the total amount awarded under the scheme.

Classification of awards by broad scientific category and ‘R’

We fund a broad range of research across the biomedical and life sciences, engineering, mathematics and computer sciences. To monitor and assess the impact of our funding schemes, we classify awards by scientific discipline, as shown in Figure 2. Awards are also classified according to the primary ‘R’ that the research aims to address (i.e. replacement, reduction or refinement).

Figure 2: Scientific classification scheme for NC3Rs awards

PHARMACEUTICALS AND CHEMICALS

- Biologics
- Ecotoxicology
- Efficacy
- Pharmacology
- Safety
- Toxicology

CELLS AND SYSTEMS

- Cancer
- Cell biology
- Developmental biology
- Genetics
- Physiology and disease

NEUROSCIENCE AND BEHAVIOUR

- Animal welfare
- Basic neurobiology
- Behaviour
- Cognition
- Neurodegenerative diseases
- Neurological disorders
- Pain and analgesia

INFECTION, IMMUNITY AND INFLAMMATION

- Basic immunology
- Host-pathogen interactions
- Infectious agents
- Immunity and immune tolerance
- Inflammation
- Vaccines

Figure 3: Studentship awards by broad scientific category and primary ‘R’, 2009 to 2016

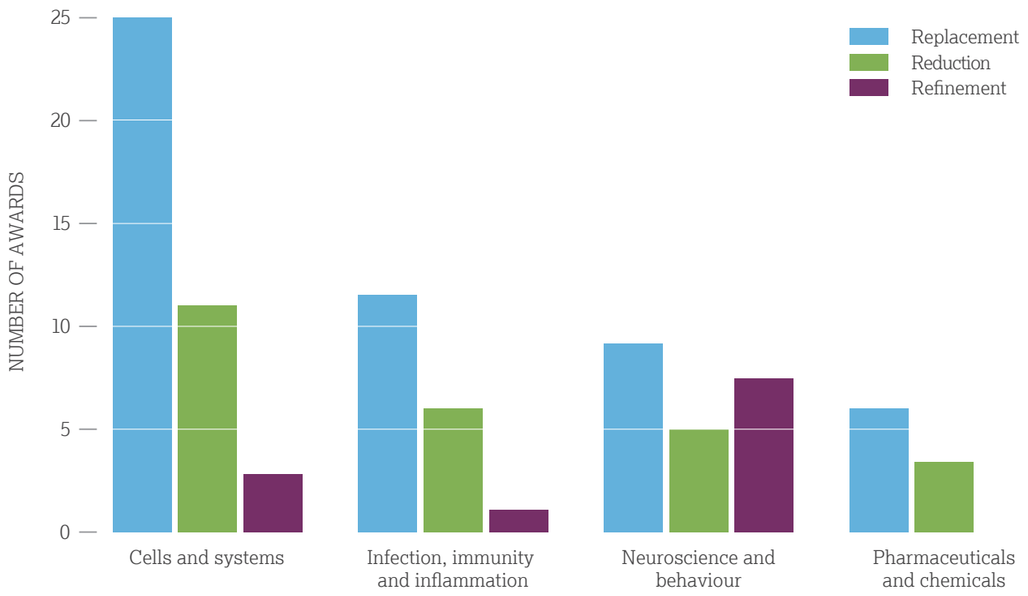
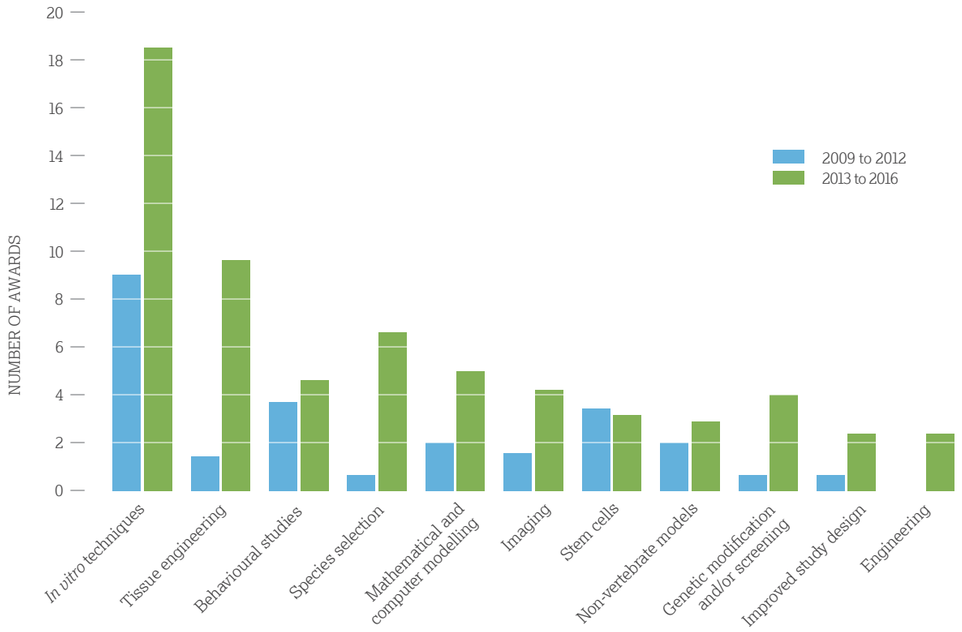


Figure 3 shows the number of studentship awards by broad scientific category and primary ‘R’. The majority of the awards are for projects aimed at replacement of animal use (58%), followed by reduction (29%) and refinement (13%). This distribution largely reflects the number of applications received in these areas and is comparable to that observed across other NC3Rs funding schemes. Awards in the cells and systems category account for 45% of the total awards made, a commitment value of £3.6 million.

Classification of awards by key technologies and approaches

Figure 4: Technologies and approaches developed, or used in, NC3Rs studentship awards*, 2009 to 2016



*Awards may involve more than one technology or approach, in which case the weighted average has been used.

A wide range of technologies and approaches have been developed or employed through the studentship awards, as illustrated in Figure 4. The number of awards utilising *in vitro* techniques, tissue engineered models, and genetic modification and/or screening has risen in recent years, due in part to the development of new cell culture and gene editing techniques and biomaterials. In addition, the increasing acceptance by the research community of models using embryonic life forms such as zebrafish larvae and chick embryos has led to an increase in the number of awards in the species selection category. There has also been an increase in the number of awards that include mathematical and computational modelling, and imaging. This has been stimulated by two strategic calls in these areas in 2012 and 2013 respectively.

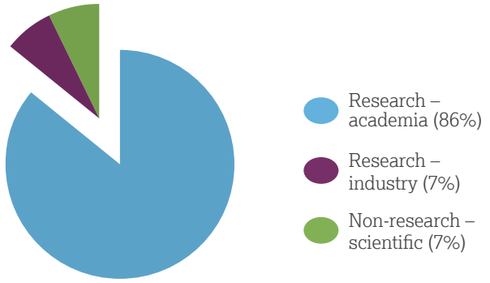
Student progression and next destination

The ten students who began their PhDs in 2009 and 2010 have completed their studies, with some of the students who started in 2011 having completed as well.

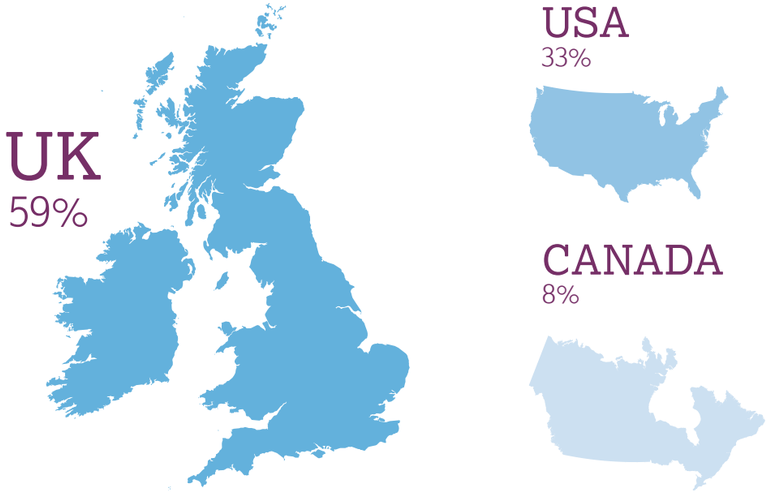
The majority (86%) of the completed students have stayed within academic research, going on to take up postdoctoral positions in UK and North American universities (Figure 5).

Students have also successfully transitioned to other research intensive fields in industry and other sectors.

Figure 5: Next destination of students who have completed their PhD studentship



The 86% of students who have stayed within academic research have obtained positions in the following countries:



Publications

Publications, particularly first author papers, are important for the development of the student's future career in research. 71% of NC3Rs students finish their PhD studies with at least one publication. On average, NC3Rs-funded students who have finished their PhD have published two papers, with an average of one first author paper per student.

Table 2 shows the number of publications from NC3Rs PhD students up to 2015 based on data collected from Researchfish.

Publications from the students highlighted in the case studies in this review are listed in Appendix 1.

Table 2: Number of publications* arising from studentship awards by whole calendar year, 2011 to 2015

	2011	2012	2013	2014	2015	Total	Total first author
Research papers	0	1	1	14	11	27	13
Reviews	1	0	0	4	6	11	8
Other	0	0	0	1	1	2	0

*All research papers and reviews are peer reviewed. 'Other' includes peer reviewed and non-peer reviewed papers or book chapters. Publications from 2016 have not been included as they will be collated following the next Researchfish data submission period in 2017.

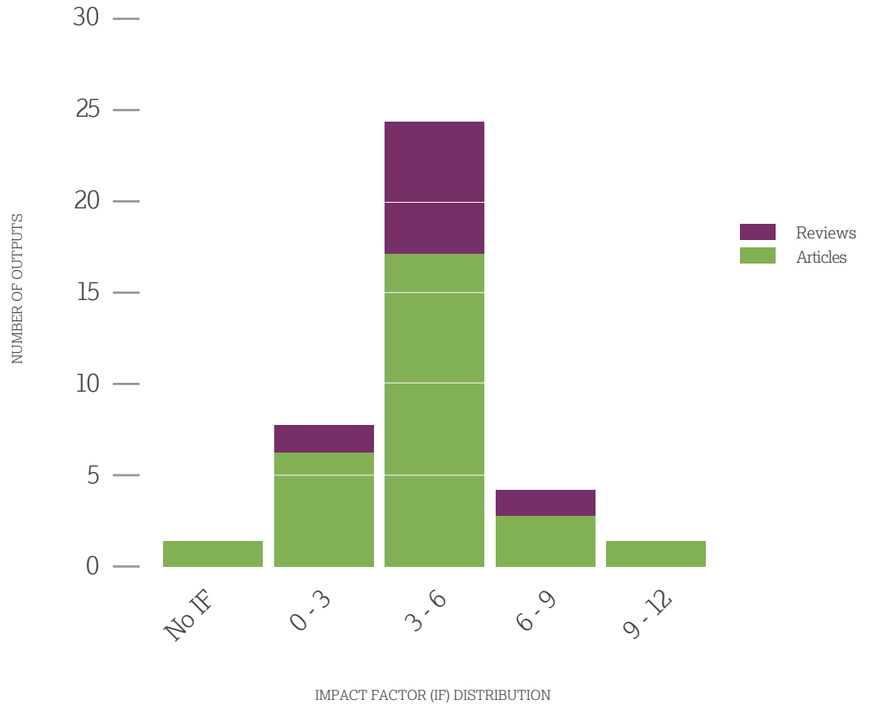
The number of publications increased in 2014, coinciding with the completion of the first cohort of three and four-year studentships awarded in 2009.

As most publications arise in the final year of the studentship and beyond completion of the *viva*, publication rates are likely to fluctuate year to year depending on the number of awards made in previous years and the number of students completing their studies in the given year.

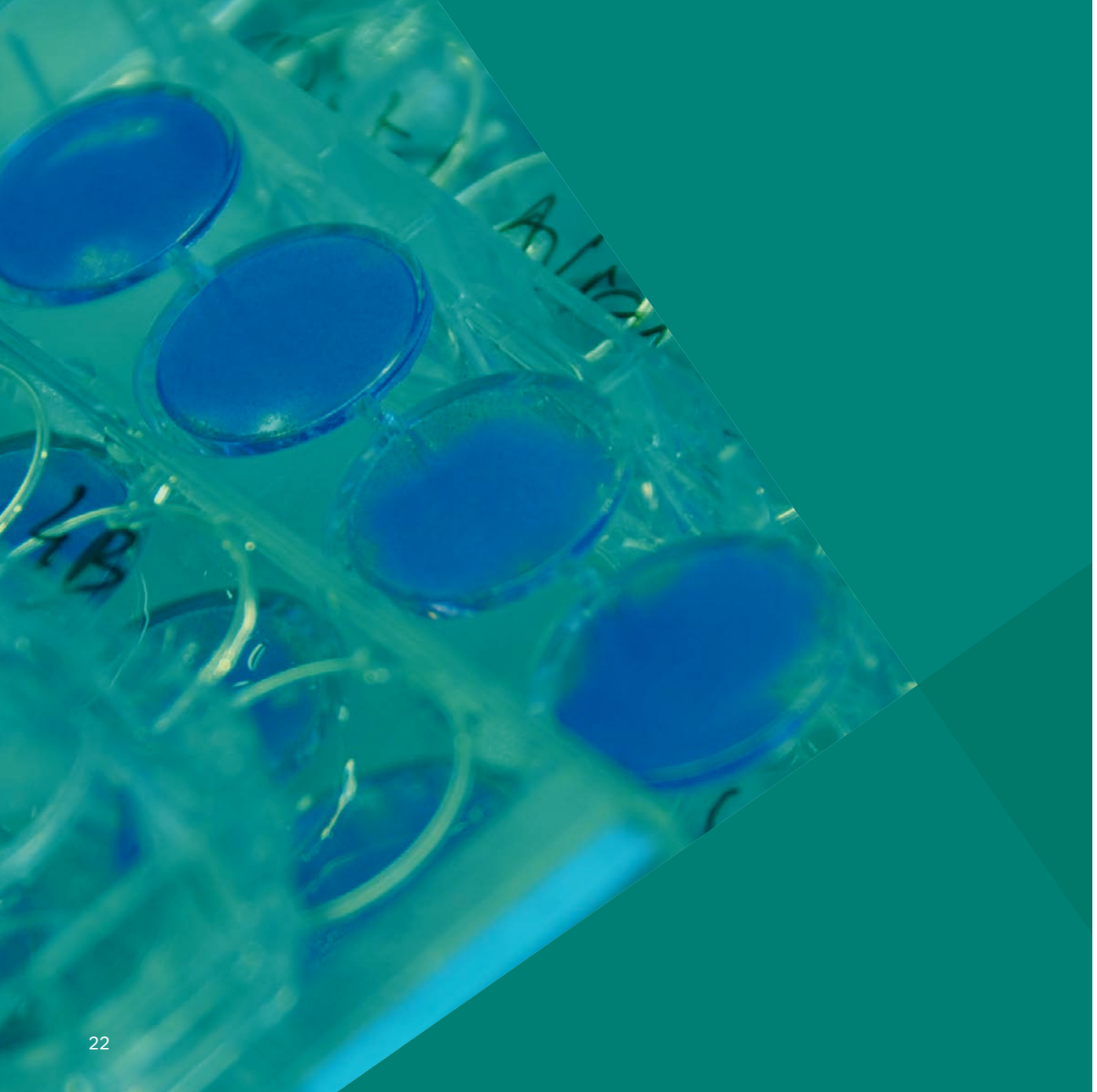
The quality of papers is most often measured based on the impact factors of the journals in which they are published.

The papers and reviews arising from our studentships are published primarily in journals with an impact factor of three or more, as shown in Figure 6, putting them in the top 16% of the 11,698 journals that were assigned an impact factor in 2014.

Figure 6: Journal impact factors for studentship publications, 2011 to 2015*



* Figures are accurate to whole calendar year (2011 to 2015).



Summer School

The NC3Rs Summer School is a unique event, bringing together our first year PhD students from universities across the UK with expert speakers and NC3Rs staff.

Over three days, the students gain a thorough understanding of the 3Rs and their scientific importance, learn transferable skills to help them with their studies, and network with all involved.

In recent years, we have opened up the Summer School to PhD students in the biological sciences at the host institution, to further extend our 3Rs training for junior researchers.

The 2015 School was held in collaboration with the University of Oxford, and the 2016 School in collaboration with the University of York.

The programme is varied, dynamic and fun, consisting of interactive workshops and lectures, group activities, quizzes, team building exercises and social events. There are practical sessions on planning, structuring and managing a PhD, principles of robust experimental design, the ARRIVE guidelines, communicating science to a variety of audiences, and career options in academia and industry. The students also work in groups to solve 'real world' 3Rs problems. Teams are presented with widely used animal models, for example in the fields of cancer and stroke research. They are then challenged to think creatively to develop alternative approaches that overcome the scientific limitations and animal welfare concerns associated with the models.

The Summer School is the first time that the NC3Rs-funded students come together as a cohort, and it provides an excellent opportunity to establish, together with NC3Rs staff, a strong and supportive network of 3Rs-minded researchers. Working alongside staff and fellow students, the attendees learn more about the role and work of the NC3Rs, and the ways in which we can help them to achieve 3Rs impact from their research.

We also provide the tools to help maintain the relationships formed at the event, including a dedicated LinkedIn group and encouraging regular attendance at NC3Rs symposia and workshops.

Case studies

Our studentship awards not only train early career scientists in the fundamental principles of the 3Rs, they also achieve real impacts in terms of reductions in animal use and improvements in animal welfare.

Our students have developed a wide range of novel 3Rs technologies which are now being used by academic and commercial laboratories across the country and internationally. In addition to the generation of new research materials, supervisors and students have secured additional funding for continuing their research. Our students have also been internationally recognised, and have received awards and prizes in a broad range of fields, from basic science through to translational medicine and technology.

From left to right:
Dr James Dear
Bastiaan Vliegenthart

3Rs approaches to assessing hepatotoxicity in drug development

Student:
Bastiaan Vliegenthart

Principal Investigator:
Dr James Dear

Co-supervisors:
Dr Matthew Bailey
Professor David Webb

Organisation:
University of Edinburgh

Bastiaan Vliegenthart started his PhD studentship at the University of Edinburgh in 2013 under the primary supervision of Dr James Dear.

Hepatotoxicity is assessed during the development of new drugs, often using animals. Studies involve histopathology, and analysis of biomarkers as read-outs of liver toxicity using various doses of the drug candidate. Depending on the chemistry of the drug candidate and its target, bespoke toxicity studies may be conducted early in the development pipeline, typically with rodents. Subsequently, liver toxicity is assessed in regulatory toxicology studies that involve rodent and non-rodent species. Hepatotoxicity is a major reason for drug attrition and post-marketing drug-induced liver injury can be a significant problem, with approved drugs being the most common cause of acute liver failure in the USA, for example.

Bastiaan has developed two methods for assessing liver toxicity using adult and larval zebrafish which have the potential to minimise the use of rodents. The first focused on miRNAs – small non-protein coding RNAs involved in post-transcriptional gene regulation. miRNAs are protected from degradation in the blood and represent a pool of biomarkers for disease and toxicity. For example, miR-122 is a highly conserved biomarker for paracetamol-induced liver toxicity in rodents and man. The limited blood volume available from standard collection methods in the zebrafish has precluded its use for the discovery and validation of such biomarkers of clinical relevance. To address this, Bastiaan developed a method for retro-orbital (RO) sampling from euthanased zebrafish which allowed the blood collected to be maximised, using 63% fewer fish than the traditional lateral incision method (13 instead of 36 fish). Using the RO technique, Bastiaan demonstrated for the first time that circulating miRNAs could be measured using a zebrafish model of paracetamol hepatotoxicity, with miR-122 elevated in a dose-dependent pattern, consistent with findings in humans and rodents.

The second method uses transgenic zebrafish larvae as a screen for potential hepatotoxic compounds, based on the observation that larval livers show patterns of injury and biomarkers comparable to that seen in mammalian liver. The transgenic zebrafish larvae express fluorescent liver-type fatty acid-binding protein (LFABP), a sensitive marker for liver cell damage. When the larvae are exposed to hepatotoxic drugs, the intensity of the fluorescence can be used as a real time indicator of liver injury. Using larvae that are double transgenic for fluorescent LFABP and a marker for macrophages and neutrophils, Bastiaan has demonstrated for the first time that the immune response to liver injury can be quantified, as the infiltration of fluorescent immune cells into the liver can be tracked in high resolution, and quantified using selective plane illumination microscopy. Dr Dear is now working with AstraZeneca to commercialise the model with funding from Medical Research Scotland. He has also secured £50k from the UK Regenerative Medicine Platform to use the model to test a range of regenerative compounds.

Bastiaan's project has also had a direct clinical element that included identifying panels of miRNAs for paracetamol overdose that can be used for patient stratification in hospital, allowing earlier prediction of those at high and low risk of liver injury. Dr Dear has partnered with Qiagen to develop this test as a diagnostic tool for A&E departments. With 48% of all poisoning admissions to UK hospitals due to paracetomol overdose, the test could have an important impact on patient outcomes.

To date, Bastiaan has published six papers, four as first author. He was awarded the Gerhard Zbinden Young Scientist Award at the 2014 EUROTOX conference.

Reducing the use of rats in memory research

Student:
Kamar Ameen-Ali

Principal Investigator:
Dr Alexander Easton

Co-supervisor:
Professor Madeline Eacott

Organisation:
Durham University

Kamar Ameen-Ali completed her PhD studentship at Durham University in 2014 under the primary supervision of Dr Alex Easton.

Spontaneous recognition memory is commonly impaired in neurodegeneration. In the laboratory, spontaneous object recognition is a memory test that uses the innate preference of animals for exploring new items over familiar items to assess their ability to remember objects (or novel configurations of objects) that they have seen before. Behaviour in these tests can be influenced by a range of factors other than novelty, including handling. This extra-experimental variance necessitates the use of a large number of animals to detect a biologically relevant effect, and the accumulation of data can be time consuming as animals usually perform only one trial a day.

Kamar designed a new experimental apparatus to test spontaneous recognition memory in rats that allows for multiple trials in a session and reduces handling stress. The apparatus is composed of two compartments, a static holding area where the animal is initially placed and where it remains before and after each trial, and the object area where the testing takes place. The object area can be changed to reveal a new context whilst the animal is secure in the holding area.

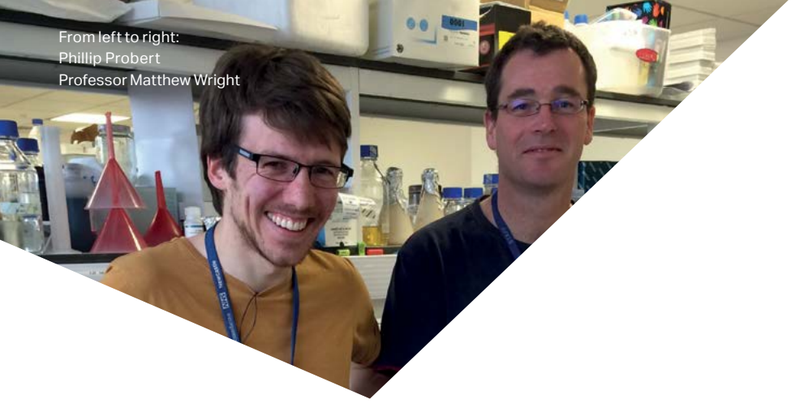
By opening and closing the doors between the two compartments, the animal can easily move between the holding and test areas without the need for handling. Using the apparatus, it is possible to reduce the number of rats used per experiment from 15 to five. Recognition tasks are widely used across a number of disciplines and the multiple trial apparatus could reduce the use of around 3,000 animals a year, based on papers published in the field.

Through the NC3Rs technology partnering hub CRACK IT Solutions, Dr Easton has collaborated with GlaxoSmithKline in Shanghai, China to modify the apparatus for use with mice. This was facilitated with £30k of seed funding from the NC3Rs plus matched funding from Durham University to allow Dr Easton to support a new PhD student. The collaboration has demonstrated that it is possible to reduce the number of mice in these types of memory studies from 16 to 20 animals per experiment to just eight. Using the apparatus, it has been demonstrated that mice, double transgenic for amyloid precursor protein and presenilin-1 mutant genes, show no biologically significant impairment in common tasks of memory, suggesting that previous observations to the contrary could be due to anxiety induced by handling.

In addition to two first author papers, Kamar has presented her research to MPs and other invited guests at the final of the 2013 Science, Engineering and Technology (SET) for BRITAIN event, held in the House of Commons. Following her PhD, Kamar worked as a postdoctoral researcher at the University of Sheffield, bringing her behavioural expertise to a laboratory working on rodent Alzheimer's disease models.

Reduction of 3,000 animals a year

In December 2016, Kamar joined the NC3Rs as a Regional Programme Manager working with the Universities of Manchester, Liverpool and Sheffield to help support implementation of the 3Rs on the ground.



From left to right:
Phillip Probert
Professor Matthew Wright

Refining liver fibrosis studies in rodents

Student:
Phillip Probert

Principal Investigator:
Professor Matthew Wright

Co-supervisors:
Professor Derek Mann
Dr Fiona Oakley

Organisation:
Newcastle University

Phillip Probert completed his PhD studentship at Newcastle University in 2015 under the primary supervision of Professor Matthew Wright.

Chronic liver disease is the fifth leading cause of mortality in the UK, and the only one that is continuing to rise year on year. Diseases such as Hepatitis B and C, and non-alcoholic fatty liver disease often lead to injury of the liver, which causes fibrosis. This scar tissue reduces the liver's ability to function and to regenerate after injury. When large amounts of scar tissue begin to replace healthy tissue – a condition known as cirrhosis – liver failure and death can occur. Despite years of research, much of it involving animals, there are currently no therapeutics available to prevent or treat liver fibrosis and cirrhosis, with liver transplantation being the only option.

One of the most commonly used experimental models for liver fibrosis involves surgical ligation of the bile duct in mice or rats to induce hepatic periportal fibrosis. This procedure is irreversible, and classified as severe under the UK's Animals (Scientific Procedures) Act 1986 because of its impact on animal welfare and the high risk of surgery-associated complications including death.

Phillip developed two refined methods of inducing liver fibrosis that do not require surgery and are classified as moderate procedures. They involve orally dosing rodents with either methylpyrilene or α-naphthylisothiocyanate. These hepatotoxins cause liver damage similar to that caused by the bile duct ligation procedure, but are more reflective of human disease, as the damage is reversible and the severity of the damage can be modulated to simulate different stages of liver fibrosis. This allows the models to be used to test new drugs that could potentially reverse liver fibrosis. This work was published in *Toxicology Research* in 2014.

Professor Wright has now completely replaced the bile duct ligation procedure with these more refined methods within his own laboratory. The models could have a significant welfare impact on the approximately 4,500 animals a year that undergo bile duct ligation for fibrosis research worldwide.

Phillip also developed an *in vitro* replacement model using rat pancreatic cells, which can be differentiated into hepatocyte-like cells for use in screening new drugs for toxicity. A workshop was held in 2014 to promote the use of the cells, supported by the NC3Rs, the Alternatives Research & Development Foundation, and the British Toxicology Society.

Welfare impact on 4,500 animals a year

Phillip is now a postdoctoral researcher in Professor Wright's laboratory, where he is working on identifying mechanisms of mitochondrial toxicity in environmental samples using the models he developed during his PhD.

Refining handling techniques for mice

Student:
Kelly Gouveia

Principal Investigator:
Professor Jane Hurst

Organisation:
University of Liverpool

Kelly Gouveia completed her PhD studentship at the University of Liverpool in 2014 under the supervision of Professor Jane Hurst.

Different methods of handling and restraint of laboratory animals have been shown to influence their physiology and behaviour. This can affect animal welfare and introduce variability between animals that necessitates the use of larger group sizes in order to detect biologically relevant effects against the background 'noise'. It can also lead to variability between experiments which may have an impact on the reproducibility of the findings.

Kelly established a refined handling technique that involves picking mice up with a small tunnel, rather than using their tail – the most commonly used method.

Not only does tunnel handling avoid the aversion and high anxiety shown by mice picked up and restrained by the base of the tail, but it also improves the reliability of their performance on cognitive behavioural tests. For example, mice are more willing to explore the arms of a radial maze, used to assess spatial learning and memory, when handled with a tunnel rather than by the tail. They also show more reliable

investigation of stimuli in an open arena when handled with a tunnel or cupped on the open hand. Reduced exploration activity by tail handled mice in both of these situations makes it difficult to determine the basis of any changes in the animal's behaviour and failure to learn.

The refined handling technique has the potential to impact on the welfare of millions of mice used in research globally, and ensuring that there is wide dissemination of the technique and its benefits is critical to achieving this. Kelly and Professor Hurst have taught the handling technique to a wide range of researchers and animal care staff, and have collaborated with other research organisations and industry to explore the practicality of implementing improved handling as standard practice. This includes collaborative studies with Cancer Research UK to investigate the potential to improve breeding for some strains of mice through a reduction in handling stress, and AstraZeneca to explore the potential for reducing behavioural variability in experiments testing the effectiveness of new drugs.

In 2013, Professor Hurst won a BBSRC Sparking Impact award based on this research, which Kelly, in collaboration with local Named Animal Care and Welfare Officer, Mr John Waters, used to develop a tutorial demonstrating the tunnel handling technique and its scientific benefits. We have worked closely with the Liverpool team to produce a narrated online version of the tutorial to further drive dissemination and implementation.

Wide online dissemination of refined mouse handling tutorial

The tutorial was launched on the NC3Rs website in September 2016 and was viewed by over 2,000 visitors from 53 countries in the first three months.

Identifying renal toxicity using primary cell proximal tubule assays

Student:
Sarah Billington

Principal Investigator:
Dr Colin Brown

Organisation:
Newcastle University

Sarah Billington completed her PhD studentship at Newcastle University in 2015 under the supervision of Dr Colin Brown.

Accurate prediction of the safety of new drugs is a major challenge for the pharmaceutical industry. Approximately 50% of new drugs fail in the first phase of clinical trials, with around 20% of these failures due to the drugs' toxic effects on the kidney, which were not detected in preclinical screening, including in animal tests.

Sarah and her colleagues developed the aProximate™ models, primary cell-based human and rat models of the proximal tubule. Sarah optimised the cell isolation techniques for the human and rat models and characterised the cells, focusing on the expression of renal transporters that are involved in drug transport.

The cells express the full complement of endogenous renal transporters at physiological levels. This provides researchers with a novel tool to investigate drug transport in and out of the proximal tubule without using animals, and to identify nephrotoxic drugs before extensive animal toxicology studies are undertaken. Drug-drug interactions, the interaction of compounds with renal drug transporters, and mechanisms of action of a compound can all be studied in this model system.

In 2014, through the NC3Rs CRACK IT Solutions technology partnering hub, Dr Brown formed a commercial partnership with SOLVO Biotechnology to include the aProximate™ assay in their portfolio. SOLVO is a contract research organisation specialising in transporter assays for drug toxicity testing. Since then, Dr Brown's laboratory has been contracted to screen around 100 new drug molecules for many of the major pharmaceutical companies, replacing the use of thousands of animals, and generating a substantial revenue stream for Newcastle University.

Sarah also worked in partnership with pharmaceutical company Gilead to determine the mechanisms of transport for Tenofovir, an anti-retroviral medication used to treat HIV and Hepatitis B. Tenofovir is widely used due to its effectiveness and ease of dosing, but its long-term use can result in kidney damage and failure from toxic accumulation in the proximal tubule. Sarah demonstrated that OATP4C1, a relatively unknown renal transporter, plays a key role in the accumulation of anti-retroviral agents and other drug molecules in the kidney. This led Gilead to invest in developing assays for OATP4C1 drug-drug interactions to better predict renal toxicity of new drug molecules.

Better prediction of renal toxicity could reduce animal use

Sarah now holds a postdoctoral position at the University of Washington in Seattle, USA, where she continues her research into renal drug transporters in the field of quantitative proteomics.

Improved *in vitro* tests for genotoxicity testing

Student:
Katherine Chapman

Principal Investigator:
Professor Gareth Jenkins

Co-supervisor:
Dr Shareen Doak

Organisation:
Swansea University

Katherine Chapman completed her PhD studentship at Swansea University in 2015 under the primary supervision of Professor Gareth Jenkins.

New chemicals, including pharmaceuticals and pesticides, undergo rigorous safety assessments before they can be approved for use. This includes an assessment of their genotoxic potential because of the link between genotoxicity and the development of cancer. Genotoxicity testing is conducted in two stages. Chemical compounds are first assessed using *in vitro* models. If the chemicals test positive for genotoxicity, they are then tested in animals, primarily mice.

Thousands of mice are used in genotoxicity tests each year, many of which are unnecessary due to high rates of misleading positive *in vitro* results. A number of factors in the first stage of testing contribute to the misleading positives. This includes the use of non-human cells that have critical mutations (for example, in the *p53* gene) causing over-sensitivities to certain chemicals, and exposure of the cells to high doses of chemicals which are not reflective of human exposure. More predictive and accurate *in vitro* approaches would reduce the number of misleading positives and the number of animals used in the second stage of testing. Katherine developed two *in vitro* genotoxicity models, a 2D model and a 3D model, both using human cells. The 2D model was combined with a low dosing regimen to investigate the effects of chronic exposure on genotoxicity, a scenario which better reflects human exposure. A 3D model was also developed using primary human keratinocytes to better represent the cell-to-cell contact and signalling seen *in vivo*. This was shown to be less sensitive to the effects of chemicals than the 2D model, suggesting that if used during the first stage of genotoxicity testing, it could result in fewer misleading positives.

Professor Jenkins was awarded a £380k NC3Rs strategic award in 2011 focusing on the development of an integrated model to predict carcinogenicity. The model combines genotoxicity data with information on disruption to other cellular features such as cell signalling, division, and morphology. Part of this work builds on findings from Katherine's studies, which determined that there are important differences in chronic versus acute dosing regimens. In 2014, Professor Jenkins was also awarded funding of £60k from Unilever for a PhD studentship which further develops Katherine's 2D model. The aim of this studentship is to create a passive dosing model to mimic the slow and continuous release of chemicals from a source such as an implant.

Katherine has won a number of international awards, including the Student and New Investigator Presentation award at the 2014 Environmental Mutagenesis and Genomic Society annual conference in Florida, USA.

In 2015, she secured a £5k pilot grant from the UK Environmental Mutagenesis Society to further develop a mitochondrial toxicity assay that can recognise different classes of carcinogens and non-genotoxic chemicals.

£445k
in further funding

Katherine is currently working as a postdoctoral researcher with Professor Jenkins.

An *in vitro* model for pain and neurogenic inflammation

Student:
Rebecca Clarke

Principal Investigator:
Dr Fionnuala Lundy

Co-supervisors:
Dr Tim Curtis
Dr Lorcan McGarvey

Organisation:
Queen's University Belfast

Rebecca Clarke completed her PhD studentship at Queen's University Belfast in 2014 under the primary supervision of Dr Fionnuala Lundy.

The oro-facial region and upper airway is innervated by the trigeminal nerve, a large and complex sensory nerve with specialised receptors that transduce environmental stimuli into pain sensation and protective airway reflexes. The largest group of these receptors is the Transient Receptor Potential (TRP) channel family, and as such, they are a major focus in the development of analgesics. Tissue damage and inflammation in the oral cavity and upper airway results in the release of inflammatory mediators such as bradykinin and prostaglandins from the damaged tissues, stimulating the TRP receptors and causing sensitisation of the nerves. This neural sensitisation results in a heightened nociceptive response to painful stimuli, and the sensation of pain in response to normally innocuous stimuli. Sensitisation can persist long after the initial tissue damage or inflammation has subsided, resulting in neurogenic pain, where pain is experienced in the absence of direct nociceptor stimulation by trauma or disease. Neurogenic pain and inflammation is a complex, chronic condition which is modelled in a number of different animals including mice, rats, guinea pigs, and ferrets. Studies primarily involve nerve injury, for example by axotomy or chemical damage. However, there are questions about the utility of the data obtained and its clinical translation, with neurogenic pain and inflammation continuing to be areas of unmet medical need.

Rebecca developed an *in vitro* neurogenic pain and inflammation model using human peripheral nerve cells cultivated from dental pulp from teeth extracted as part of routine dental procedures. A significant advantage of the model is that it offers the ability to build complex co-cultures, including other cell types such as leucocytes and primary epithelial cells, to provide a more biologically realistic model. Rebecca focused on applying the model to the study of the upper airway, where neural sensitisation is often caused by respiratory infections. This included testing the efficacy of treatments for cough, supported by a €50k award from the phytopharmaceutical company, Bionorica.

When the sensory nerve cells in the upper airway are sensitised by inflammation as a result of common cold viruses, this can cause a hyper-responsive cough reflex which can persist for weeks after the infection has cleared, causing an irritating cough typically triggered by stimuli which would not normally irritate the airway, such as changes in ambient air temperature, talking or laughing. Rebecca was able to use the model to show that specific compounds provided by Bionorica could restore a normo-responsive state in nerves that she had rendered hyper-responsive by pre-treatment with cytokines or the viral mimic polyinosinic:polycytidylic acid, without suppressing the healthy cough reflex.

Rebecca has won a number of awards for her research. In 2013, this included the award for best oral abstract at the American Cough Conference in New York, and a Hatton award at the Irish Division of the International Association for Dental Research (IADR) annual meeting. The latter is sponsored by Unilever and allowed Rebecca to present her research at the 2014 IADR General Session in Cape Town, South Africa.

Winner of the Bionorica Global Research Initiative award (€50k)

Rebecca completed her PhD in 2014, and is now working at Momentum Bioscience in Oxford to develop diagnostic products to help combat global antibiotic resistance.

Reducing the use of the intravenous mouse model for studies of systemic *Candida albicans* infection

Student:
Edina Szabo

Principal Investigator:
Dr Donna MacCallum

Co-supervisor:
Dr Carol Munro

Organisation:
University of Aberdeen

Edina Szabo completed her PhD studentship at the University of Aberdeen in 2014 under the primary supervision of Dr Donna MacCallum.

Candida albicans is a commensal organism that can cause life-threatening systemic infections in severely ill patients, with mortality rates of around 40%. The mouse intravenous challenge model is commonly used to study systemic candidiasis in the laboratory. In this model, fungal cells spread throughout the body, and although infection is controlled in most organs, it progresses in the kidneys and leads to sepsis and death. The model is therefore associated with a high degree of suffering and is classified as severe under the Animals (Scientific Procedures) Act 1986. Edina developed a mouse renal epithelial cell model to assay kidney/pathogen infections for *C.albicans*. The model is based on findings that fungal burden in the kidney is accompanied by increasing levels of renal cytokines and chemokines, and that renal cytokine levels correlate with lesion severity and eventual infection outcome. The epithelial cell assay was shown to respond only to live *C.albicans* cells capable of forming hyphae, and to produce chemokines with levels correlating with epithelial cell damage. Importantly, comparison with clinical isolates of known virulence in the murine intravenous challenge model demonstrated that the renal epithelium assay can differentiate between attenuated and virulent strains, thus providing an alternative to the mouse systemic infection model.

The work was published in *Virulence* in 2014 and the model has since been taken up by other groups within the MRC Centre for Medical Mycology at the University of Aberdeen, reducing rodent use by around 50 animals per year. Edina has three publications and is now a postdoctoral researcher at the University of Calgary in Canada working on the impact of *Heligmosomoides polygyrus* worm infections on innate immune responses to the single-celled intracellular parasite *Toxoplasma gondii*.

Dr MacCallum has subsequently been awarded an NC3Rs pilot study grant to develop near-infrared reporters for *C.albicans* that allow real time imaging of systemic candidiasis to be tracked deep within the mouse's organs.

Temporal studies of infection typically involve the use of up to ten animals per time point because of inter-animal variability. The ability to monitor infection longitudinally in the same animal could reduce sample sizes by 75%. In 2015, Dr MacCallum was awarded further NC3Rs PhD studentship funding to develop comparable reporters for *Aspergillus fumigatus* and *Cryptococcus neoforman*, both of which can cause serious infections in immunodeficient individuals.

Replaced severe mouse model of systemic infection with an *in vitro* assay

As a result of Dr MacCallum's NC3Rs awards, she has been appointed to the university's Animal Welfare and Ethical Review Body, and chairs a university-wide 3Rs committee.

Appendix 1: Publications from the case studies

Dr James Dear - Bastiaan Vliegenthart

Vliegenthart AD, Starkey Lewis P, Tucker CS, Del Pozo J, Rider S, Antoine DJ, Dubost V, Westphal M, Moulin P, Bailey MA, Moggs JG, Goldring CE, Park BK, Dear JW (2014). Retro-orbital blood acquisition facilitates circulating microRNA measurement in zebrafish with paracetamol hepatotoxicity. *Zebrafish* 11(3):219-26. doi: 10.1089/zeb.2013.0912

Vliegenthart AD, Tucker CS, Del Pozo J, Dear JW (2014). Zebrafish as model organisms for studying drug-induced liver injury. *British Journal of Clinical Pharmacology* 78(6):1217-27. doi: 10.1111/bcp.12408

Vliegenthart AD, Shaffer JM, Clarke JI, Peeters LE, Caporali A, Bateman DN, Wood DM, Dargan PI, Craig DG, Moore JK, Thompson AI, Henderson NC, Webb DJ, Sharkey J, Antoine DJ, Park BK, Bailey MA, Lader E, Simpson KJ, Dear JW (2015). Comprehensive microRNA profiling in acetaminophen toxicity identifies novel circulating biomarkers for human liver and kidney injury. *Scientific Reports* 5:15501. doi: 10.1038/srep15501

Liga A, Vliegenthart A D B, Oosthuyzen W, Dear J W, Kersaudy-Kerhoas M (2015). Exosome isolation: a microfluidic road-map. *Lab on a Chip* 15(11):2388-94. doi: 10.1039/c5lc00240k

Vliegenthart AD, Antoine DJ, Dear JW (2015). Target biomarker profile for the clinical management of paracetamol overdose. *British Journal of Clinical Pharmacology* 80(3):351-362. doi: 10.1111/bcp.12699

McCrae JC, Sharkey N, Webb DJ, Vliegenthart AD, Dear JW (2016). Ethanol consumption produces a small increase in circulating miR-122 in healthy individuals. *Clinical Toxicology* 54(1):53-5. doi: 10.3109/15563650.2015.1112015

Dr Alexander Easton - Kamar Ameen-Ali

Ameen-Ali KE, Eacott MJ, Easton A (2012). A new behavioural apparatus to reduce animal numbers in multiple types of spontaneous object recognition paradigms in rats. *Journal of Neuroscience Methods* 211(1): 66- 76. doi: 10.1016/j.jneumeth.2012.08.006

Ameen-Ali KE, Easton A, Eacott MJ (2015). Moving beyond standard procedures to assess spontaneous recognition memory. *Neuroscience and Biobehavioral Reviews* 53:37-51. doi: 10.1016/j.neubiorev.2015.03.013

Professor Jane Hurst - Kelly Gouveia

Gouveia K, Hurst JL (2013). Reducing mouse anxiety during handling: effect of experience with handling tunnels. *PLoS One* 8(6):e66401. doi: 10.1371/journal.pone.0066401

Link to online mouse handling video tutorial: https://www.nc3rs.org.uk/mouse-handling-tutorial

Professor Gareth Jenkins - Katherine Chapman

Seager AL, Shah UK, Brüsehafer K, Wills J, Manshian B, Chapman KE, Thomas AD, Scott AD, Doherty AT, Doak SH, Johnson GE, Jenkins GJ (2014). Recommendations, evaluation and validation of a semi-automated, fluorescent-based scoring protocol for micronucleus testing in human cells. *Mutagenesis* 29(3):155-164. doi: 10.1093/mutage/geu008

Chapman KE, Thomas AD, Wills JW, Pfuhler S, Doak SH, Jenkins GJ (2014). Automation and validation of micronucleus detection in the 3D EpiDerm™ human reconstructed skin assay and correlation with 2D dose responses. *Mutagenesis* 29(3):165-75. doi: 10.1093/mutage/geu011

Chapman KE, Doak SH, Jenkins GJ (2015). Acute dosing and p53-deficiency promote cellular sensitivity to DNA methylating agents. *Toxicological Sciences* 14(2):357-365. doi: 10.1093/toxsci/kfv004

Dr Donna MacCallum - Edina Szabo

Szabo EK, MacCallum DM (2011). The contribution of mouse models to our understanding of systemic candidiasis. *FEMS Microbiology Letters* 320(1):1-8. doi: 10.1111/j.1574-6968.2011.02262.x

Szabo EK, MacCallum DM (2014). A novel renal epithelial cell *in vitro* assay to assess *Candida albicans* virulence. *Virulence* 5(2): 286-96. doi: 10.4161/viru.27046

Mackie J, Szabo EK, Urgast DS, Ballou ER, Childers DS, MacCallum DM, Feldmann J, Brown AJ (2016). Host-Imposed Copper Poisoning Impacts Fungal Micronutrient Acquisition during Systemic *Candida albicans* Infections. *PLoS One* 11(6):e0158683. doi: 10.1371/journal.pone.0158683.

Professor Matthew Wright - Phillip Probert

Probert PME, Ebrahimkhani MR, Oakley F, Mann J, Burt AD, Manna DA, Wright MC (2014). A reversible model for periportal fibrosis and a refined alternative to bile duct ligation. *Toxicology Research* 3:98-109. doi: 10.1039/C3TX50069A

Probert PM, Chung GW, Cockell SJ, Agius L, Mosesso P, White SA, Oakley F, Brown CD, Wright MC (2014). Utility of B-13 progenitor-derived hepatocytes in hepatotoxicity and genotoxicity studies. *Toxicological Sciences* 137(2):350-70. doi: 10.1093/toxsci/kft258

Probert PME, Meyer SK, Alsaeedi F, Axon AA, Fairhall EA, Wallace K, Charles M, Oakley F, Jowsey PA, Blaina PG, Wright MC (2015). An expandable donor-free supply of functional hepatocytes for toxicology. *Toxicology Research* 4:203-22. doi: 10.1039/C4TX00214H

Richter M, Fairhall EA, Hoffmann SA, Tröbs S, Knöspel F, Probert PME, Oakley F, Stroux A, Wright MC, Zeilinger K (2015). Pancreatic progenitor-derived hepatocytes are viable and functional in a 3D high density culture system. *Toxicology Research* 5:278-90. doi: 10.1039/C5TX00187K

Amer AO, Probert PM, Dunn M, Knight M, Vallance AE, Flecknell PA, Oakley F, Cameron I, White SA, Blain PG, Wright MC (2015). Sustained isoprostane E2 elevation, inflammation and fibrosis after acute ischaemia-reperfusion injury are reduced by pregnane X receptor activation. *PLoS One* 10(8): e0136173. doi: 10.1371/journal.pone.0136173

Probert PM, Palmer JM, Alhusainy W, Amer AO, Rietjens IM, White SA, Jones DE, Wright MC (2016). Progenitor-derived hepatocyte-like (B-13/H) cells metabolise 1'-hydroxyestragole to a genotoxic species via a SULT2B1-dependent mechanism. *Toxicology Letters* 243:98-110. doi: 10.1016/j.toxlet.2015.12.010

NC3Rs
Gibbs Building
215 Euston Road
London NW1 2BE

T +44 (0)20 7611 2233
F +44 (0)20 7611 2260
enquiries@nc3rs.org.uk
twitter: @nc3rs

www.nc3rs.org.uk
www.crackit.org.uk

The material used in this publication is derived from sustainable and renewable sources. The Paper is FSC® and ISO14001 accredited. All inks used are vegetable based and all pulp is Elemental Chlorine free (ECF)