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

Annual Report 2017

Pioneering Better Science

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

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Foreword

Over the years we have demonstrated the benefits a 3Rs focus can have, not only on animal use but also on scientific discoveries and commercial opportunities.

Nevertheless, there is much more to do to ensure these benefits are fully appreciated and realised. 2017 saw the publication of our three-year strategy, with a focus on bridging the gap between the development of 3Rs approaches and their adoption into routine practice – our so-called 3Rs 'valley of death'. We recognise that driving scientific and technological advances in the 3Rs is just the first step. This needs to be matched with efforts to build confidence in, and raise awareness of, the increasingly wide range of 3Rs approaches available to encourage the shift from long-standing animal models and procedures that the NC3Rs was established to deliver.

During the last 12 months we have laid foundations for tackling the valley of death. These include new partnerships, such as that with *F1000Research* providing a dedicated platform for NC3Rs-funded researchers to publish detailed 3Rs methodologies; new funding schemes, such as the Skills and Knowledge Transfer awards for de-risking the adoption of 3Rs approaches; and new appointments of Regional Programme Managers in affiliation with universities committed to the 3Rs, who offer 'on-the-ground support' to researchers at these institutions.

We have continued to invest in high quality 3Rs R&D through our response mode funding schemes and CRACK IT programmes with a total of over £7 million committed in 2017. Supporting early career researchers remains high on our list of priorities and we were delighted to publish in the spring a review of the research from a selection of our outstanding PhD students.

Leading projects nationally and internationally, our staff often focus on data sharing and acting as an honest broker, a theme that also continued in 2017. After 11 years the Fixed Concentration Procedure for inhalation toxicology was finally accepted as an OECD Test Guideline. Still more work is needed to ensure the procedure is used in practice, highlighting both the commitment and tenacity required from any organisation working in this area and the need for a global re-think of the evidence base required for regulatory acceptance, as well as the process for achieving it.

Finally, the success of the NC3Rs is dependent on the organisations and individuals who actively participate in our work and we would like to take the opportunity to thank all of those who fund the NC3Rs, provide in-kind contributions, or who readily give their time and expertise to participate in funding panels and working groups for example. We are also enormously grateful to the MRC and BBSRC, our major funders.

Dr Vicky Robinson CBE
NC3Rs Chief Executive

Professor Stephen Holgate CBE
NC3Rs Board Chairman

UK Highlights

The Regional Programme Manager scheme: Supporting institutions to actively promote the 3Rs

A key theme described in the NC3Rs Ten Year Vision was the need to help promote and implement the 3Rs in universities. To facilitate this, we have appointed Regional Programme Managers who individually support a small number of universities.

Throughout 2017, the Regional Programme Managers have been providing dedicated 3Rs expertise to researchers and animal facility staff, helping to exchange information between institutions, promote NC3Rs funding schemes and address priority 3Rs areas identified by their universities. A range of 3Rs symposia and workshops have been organised with more than 400 delegates to highlight and share excellent 3Rs work locally, including dedicated events on fish welfare, mouse housing and chronic implants.

Currently the Regional Programme Managers are working with the Universities of Liverpool, Manchester and Sheffield, (Dr Kamar Ameen-Ali) and Birmingham, Leicester and Nottingham (Dr Emma Stringer). In 2018, the scheme will be expanded to include the Universities of Oxford, Bath, Bristol, Cardiff and Exeter, enabling researchers to easily connect to the 3Rs in these institutions. These posts are co-funded by the universities illustrating their commitment to the 3Rs.

Updating our non-human primate guidelines for accommodation, care and use

Originally published in 2006, the non-human primate (NHP) guidelines provide an overview of good practice for NHP accommodation, care and use. Compliance with the guidelines is a grant condition of many UK funding bodies, including for the work they support overseas. In 2017, the guidelines were updated taking into account new animal welfare science and addressing common issues identified through the peer review service provided by the NC3Rs for research involving NHPs. The 2017 guidelines contain additional information on experimental design and reporting, environmental enrichment, and veterinary care and welfare assessment.

The NC3Rs Gateway: a partnership with *F1000Research*

In 2017, we announced our new collaboration with *F1000Research* to develop the NC3Rs gateway. We are one of a small number of funding bodies, including Wellcome, to provide a dedicated platform for grant holders to publish their research in detail. Our gateway will ensure that all of the work funded by the NC3Rs, including methodology, negative results and 3Rs impacts, is available in the public domain promoting transparency and reproducibility. The gateway is due for launch in April 2018.

NC3Rs/ZSL Amphibian Workshop: Exploring approaches to improve amphibian welfare

There is little established guidance for the husbandry and care of amphibians, despite their growing use in research. Amphibian welfare is complex as the field is diverse with many different species used, often involving multiple life stages with differing welfare considerations. In collaboration with the Zoological Society of London (ZSL), we organised a workshop to bring together experts from the laboratory, zoological and field communities to explore approaches to help improve amphibian welfare in research. The workshop was attended by over 60 delegates and identified a number of key questions relating to the housing and husbandry of amphibians that we will be taking forward in 2018.

Global Highlights

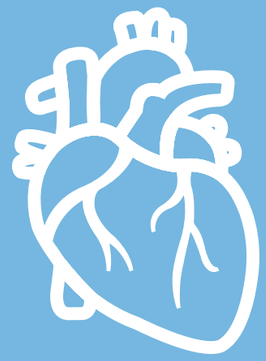
New podcast: '3 minute 3Rs'

Together with *LabAnimal*, a Nature Research journal, and the North American 3Rs Collaborative we launched a new monthly three-minute podcast summarising the latest international research and news in 3Rs science and technology. The papers are presented in a bite-size format to introduce the subjects to new audiences in an accessible way.

Supporting regulatory change: New Test Guideline adopted by OECD

The Fixed Concentration Procedure (FCP) has been adopted as OECD Test Guideline 433 for acute inhalation toxicity studies, following almost a decade of efforts led by the NC3Rs and working with colleagues from Public Health England and the Health and Safety Executive.

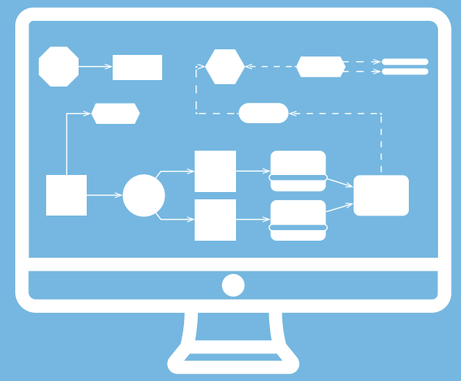
The FCP does not use death as an endpoint but relies on 'evident toxicity' (signs that predict death at the next highest concentration) and has the potential to use fewer animals than other Test Guidelines for acute inhalation toxicity. Even though an equivalent protocol existed for acute oral toxicity studies, the FCP was dropped from the OECD work plan in 2007 after some countries expressed concerns about its performance, and the subjective nature of 'evident toxicity'. We have worked since then to address these issues, carrying out a detailed retrospective analysis of inhalation toxicity data from 188 substances. This analysis enabled us to develop guidance to support the use of 'evident toxicity' and provide evidence that chemical classifications made using the FCP are comparable to those using the other Test Guidelines. We are continuing to promote the use of the FCP by working with the OECD to ensure related Guidance Documents and reporting templates are updated to include the FCP.



3Rs Prize 2017: Computer modelling can predict human cardiac safety better than animal studies

The 3Rs Prize 2017 was awarded for work describing an *in silico* 'drug trial' which predicted the risk of drug-induced heart arrhythmias in humans with higher accuracy than data obtained from previously conducted animal studies.

The work, by Dr Elisa Passini from the University of Oxford and colleagues, highlighted the great potential for computer simulations to replace animal use as well as provide meaningful human-relevant data that helps to ensure the safety of new drugs. The Panel members were impressed with the validation work done on the model to encourage uptake by the pharmaceutical industry.



Promoting the use of the experimental design resources

Responding to international demand, we participated in more than 30 events about experimental design and reporting (the EDA and ARRIVE guidelines). This included talks and workshops, both internationally (World Congress in Seattle, visits to research institutions in Brazil, India and the Netherlands) and nationally (all UK institutions where we have regional staff), as well as extensive work with funders and regulators in Europe.

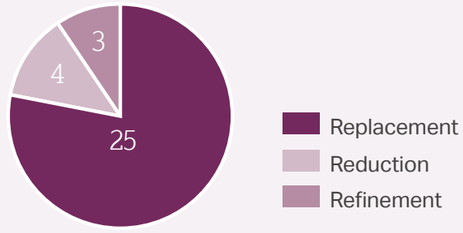
The ARRIVE guidelines are now available in eight languages, with the Korean translation published in July 2017. To improve uptake, we convened an international working group to review and revise the guidelines (see section 6 on page 22 for more details).

"The NC3Rs is a UK organisation with a global reach shaping the 3Rs landscape internationally."

2017 in numbers

1. Funding

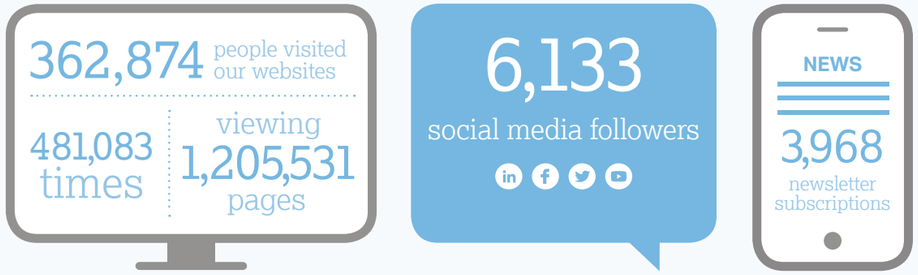
In 2017, we committed **over £5 million** in 3Rs research grants.*



*Excluding the CRACK IT programme

3Rs research grants funded by NC3Rs in 2017 by R

2. Online presence*



*Social media and subscriber figures as of December 2017. Website stats for the period January - December 2017.

3. Events

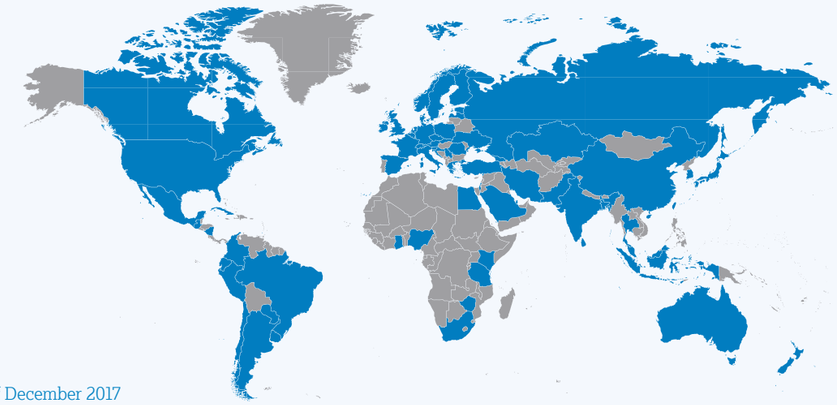
We have given 90 presentations at external events, in the UK and internationally.

We have organised 13 workshops and events with a total of 785 attendees.

4. Resources*

We have shipped over 40,000 copies of our resources to 73 countries worldwide.

Resource	
ARRIVE guidelines	17,997
Rat Grimace Scale	7,008
Mouse Grimace Scale	7,983
Rabbit Grimace Scale	5,734
Mouse Handling Posters	1,298
Total	**40,020**



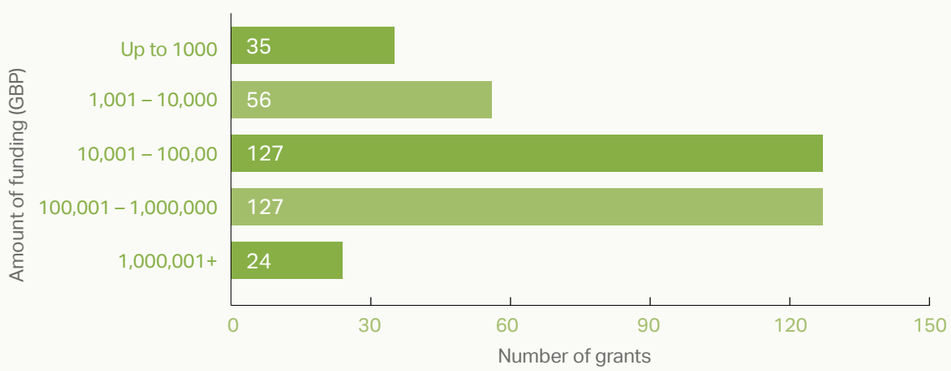
*As of December 2017

5. Researchfish*

892 publications have resulted from 161 NC3Rs awards since 2004.

127 grants reported further funding sources via Researchfish in the 2017 submission period with an average award value of £580,779.50.

Value of further funding attained by NC3Rs researchers as reported via Researchfish



*Data collected in 2017, although not relating to 2017 exclusively

Year of Laboratory Rodent Welfare

Throughout 2017, we organised a range of activities to profile the issues surrounding the welfare of laboratory rodents. We selected rodent welfare as a priority topic because of the numbers used, the nature of the procedures often involved and the opportunity to better develop and implement evidence-based refinements, including in areas where there is currently a paucity of information such as on male mouse aggression.

Mouse handling

Research by Professor Jane Hurst, University of Liverpool, shows that aversion and anxiety caused by picking up mice by the tail can be minimised by instead using a tunnel or a cupped hand. Following the release of an online mouse handling tutorial in 2016, we have been working to further the uptake of these alternative ways of picking up mice. In response to the high demand for practical tips, we produced free mouse handling posters for display in animal facilities and laboratories and organised a mouse handling workshop for trainers and named persons working under the Animals (Scientific Procedures) Act 1986. Supporting the introduction of refined handling has been a major focus for our regional staff.

"Bringing rodent welfare into focus in 2017 allowed us to demonstrate how improvements in welfare can also improve the quality of scientific outputs."

Further refinements of a mouse model of thrombosis

Dr Mike Emerson from Imperial College London studies thromboembolism using mice. With NC3Rs funding, he has previously refined the model to avoid mortality as an endpoint. However, the refined model is not widely used so Dr Emerson contacted users of the mortality model to establish the barriers to uptake. With further NC3Rs funding, he has developed a low cost and easily applied model which does not require specialist equipment, making the refinement easier to adopt.

Rauzi F, Smyth E, Emerson M (2017). Refinement of Mouse Protocols for the Study of Platelet Thromboembolic Responses *In Vivo. Thrombosis and Haemostasis* 117(12): 2283-2290. doi: 10.1160/TH17-04-0250.

Workshop at the IAT Symposium: Playtime for rats

In March 2017, we organised a workshop at the annual IAT (Institute of Animal Technology) Symposium. The session, 'Playtime for rats', was prepared in collaboration with AstraZeneca.

The workshop included presentations showcasing rats' natural needs and examples of how to cater for them in the laboratory setting both in academia and industry.

Dr Joanna Makowska, University of British Columbia, presented her 2016 3Rs prize-winning research and Dr Manuel Berdoy, University of Oxford described his work on moving rats into a semi-wild environment. The session concluded with group discussions on barriers to improved housing for rats and possible practical solutions.

Reducing variability in experimental stroke models

A study led by Dr Claire Gibson at the University of Leicester describes a new surgical approach for inducing stroke in mice that reduces variability in the experimental model, and therefore the number of animals used in a study.

The group were also awarded a Skills and Knowledge Transfer grant to help dissemination of this approach to the wider stroke community in the UK, Europe and USA.

Trotman-Lucas M, Kelly ME, Janus J, Fern R, Gibson CL (2017). An alternative surgical approach reduces variability following filament induction of experimental stroke in mice. *Disease Models & Mechanisms* 10: 931-938 doi:10.1242/dmm.029108.

TaiNi: Not so tiny breakthrough in refined neural recording in mice

As an outcome of a CRACK IT Challenge, an ultra-lightweight wireless device, TaiNi, was developed for recording neural activity in the brains of mice that avoids many welfare concerns associated with existing approaches[1]. The device was covered in the national press and a company has been established to manufacture and market it.

The Challenge, sponsored by the pharmaceutical company Lilly and funded by the NC3Rs, was to develop an EEG recording device that could be used in awake mice without the use of restraint. The competition was won by Professor Esther Rodriguez Villegas from Imperial College London, who was awarded £500,000. The final product, TaiNi, exceeded the expectations set out in the original Challenge in terms of size, weight (1.5g instead of 3g), battery life and signal bandwidth.

TaiNi offers significant animal welfare benefits including maintaining the normal repertoire of movement and behaviours, which are not possible with other wireless or tethered systems. It also has the potential to be transformative both in pharmaceutical and academic research for the study of neural networks.

Rodent Big Brother

An automated home cage monitoring system for rats was developed through the CRACK IT Challenges competition, in a Challenge sponsored by AstraZeneca and funded by the NC3Rs. The system allows, for the first time, the recording and analysis of the behaviour of individual rats socially-housed in their home cage. The validation study, published in *PLOS ONE*, confirmed the system's ability to reliably detect naturally occurring behaviours and highlighted its potential applications including safety pharmacology, toxicology, circadian biology, disease models and drug discovery.

Redfern WS *et al.* (2017). Automated recording of home cage activity and temperature of individual rats housed in social groups: The Rodent Big Brother project. *PLOS ONE* doi.org/10.1371/journal.pone.0181068.

[1]Zhou Jiang *et al.* (2017) TaiNi: Maximizing research output whilst improving animals' welfare in neurophysiology experiments. *Scientific Reports* doi:10.1038/s41598-017-08078-8.

Improving the quality of science through better animal welfare

In 2017, we published our strategy for improving the welfare of animals used in scientific research in a special edition of *Lab Animal*[1]. Our strategy for refinement includes all vertebrate species that are used and this is reflected in our portfolio of grants we award in this area. In 2017, NC3Rs grant holders published over 20 papers on improving animal welfare.

Three are highlighted here:

Reducing the use and refining the distribution of male *Xenopus*

In their publication in *Theriogenology*, Professor Matthew Guille from University of Portsmouth and colleagues optimised a sperm preservation technique for *X. laevis*. Cryogenic storage to preserve germ cells or embryos avoids the need for transporting live frogs or for keeping large numbers in stock centres. Funded by an NC3Rs Infrastructure for Impact award, the publication also shows that dividing the sperm into multiple aliquots, up to eight or more per testis, has little impact on the viability of the sperm or subsequent development, offering an opportunity for reducing the number of animals used.

Pearl E *et al*. (2017). An optimized method for cryogenic storage of *Xenopus* sperm to maximise the effectiveness of research using genetically altered frogs. *Theriogenology.* doi.org/10.1016/j.theriogenology.2017.01.007.

[1]Prescott M, Lidster K (2017). Improving quality of science through better animal welfare: the NC3Rs strategy. *Lab Animal.* doi.org/10.1038/laban.1217.

A novel automated system for positive reinforcement training of macaque monkeys

Positive reinforcement training presents a significant refinement in the training of NHPs by rewarding for desired behaviour. Dr Andrew Jackson at Newcastle University and colleagues demonstrate the use of a system that allows unsupervised and voluntary training of macaques in their home cages. The animals achieved a high level of performance with minimal staff time and without the need for food or fluid control. This study also highlights the benefits of using the system at a breeding facility rather than training upon arrival at the research facility.

Tulip J *et al. (2017).* An automated system for positive reinforcement training of group-housed macaque monkeys at breeding and research facilities. *Journal of Neuroscience Methods.* doi.org/10.1016/j.jneumeth.2017.04.015.

Social housing aids zebrafish recovery from stressors

A study from Dr Lynne Sneddon, University of Liverpool, demonstrates that group-housed zebrafish show lower levels of stress and anxiety when they undergo stressful or painful procedures. These include anaesthesia and fin clipping, both common procedures in zebrafish research. The fish resumed normal behaviour more rapidly when group-housed, and lower stress levels were also confirmed by measuring stress hormone (cortisol) levels using non-invasive methods.

White I *et al*. (2017). The impact of social context on behaviour and the recovery from welfare challenges in zebrafish, *Danio rerio. Animal Behaviour.* doi.org/10.1016/j.anbehav.2017.08.017.

Enabling uptake of 3Rs methods

We work closely with the research community to drive the uptake of 3Rs approaches, helping to ensure new developments are translated into everyday practice. We engage with a wide range of experts who help determine areas that hold opportunities for improvement. Our strategy includes a range of activities, from new funding schemes to working with stakeholders to create evidence-based guidance of best practice, developing resources, and providing free training and events.

The IMPROVE guidelines

Stroke research heavily relies on the use of rodent models. The models typically involve the blocking of a major cerebral artery and can cause significant suffering to the animals. In response to requests from the stroke research community, we convened an expert working group including stroke researchers, veterinarians and animal care staff. Based on a review of the literature and their own expertise, the working group identified opportunities to improve animal welfare and reduce variability in the approaches. The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of *in Vivo* Experiments) were published in August 2017 in the *Journal of Cerebral Blood Flow & Metabolism*, where they are currently the second most read paper with more than 2,500 views and downloads[1].

The recommendations cover basic requirements pre-surgery, selecting the most appropriate anaesthetic and analgesic regimen, as well as intra-operative and post-operative care. To increase accessibility, the guidelines were summarised in a downloadable poster, with a table of signs to monitor after experimental stroke surgery.

Launch of SyRF

In collaboration with the University of Edinburgh team led by Professor Malcolm Macleod, we launched the Systematic Review Facility (SyRF) web app – a free-to-use tool to help researchers perform systematic reviews and meta-analyses of animal studies. SyRF was developed by the Macleod group using an NC3Rs Infrastructure for Impact award.

Although systematic reviews are common practice in clinical research, they remain relatively under-utilised in animal research. There are a number of ways by which systematic reviews can advance the 3Rs, for example by supporting a reduction in animal numbers, determining whether high severity tests or multiple tests are necessary, and avoiding the use of uninformative models in animal research.

"In our three-year strategy for 2017 to 2019 we highlighted the importance of bridging the 3Rs 'valley of death'. Continued uptake and use of 3Rs methods is crucial for obtaining mature 3Rs impacts."

[1]Percie du Sert N *et al. (2017).* The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of *in Vivo* Experiments*). J Cereb Blood Flow Metab,* doi:10.1177/0271678X17709185

Skills and Knowledge Transfer grants

In 2017 we introduced the Skills and Knowledge Transfer grants to accelerate the uptake of established 3Rs technologies and approaches, bridging the gap between their development and adoption into routine use.

The awards can cover exchange visits to other laboratories, equipment and consumable costs to facilitate the transfer of new 3Rs methods and technologies between laboratories and institutions, and workshops and other resources that address wider training requirements.

Awards of up to £75k are available each year. In the first competition we awarded four grants, totalling just over £289k.

Out of the box, into the egg

Drs Inés Moreno-Jiménez and Richard Oreffo from the University of Southampton study bone regeneration and repair in the extra-embryonic membrane of a developing chick egg. The assay is minimally invasive as the membrane has blood supply but is not innervated.

Bone samples incubated on the egg membrane show signs of regeneration, making this approach a potentially useful model for studying the effects of drugs and different biomaterials on bone repair without the use of rodents.

Moreno-Jiménez I *et al*. (2016). The chorioallantoic membrane (CAM) assay for the study of human bone regeneration: a refinement animal model for tissue engineering. *Scientific Reports* 6: 32168 doi:10.1038/srep32168.

Human tissue funding highlight notice

Human tissue and cells can help reduce the reliance on animal models and increase the relevance of preclinical models to human disease. Despite these advantages, approaches involving the use of human tissue (which can include surgical discard, tissue not suitable for transplant and post-mortem material) are still relatively unexplored.

Working with researchers from academia and industry, international regulatory agencies and other key stakeholders, we have embarked on a programme of work to explore, understand and address the barriers to increased human tissue use in research.

In 2017 we launched a highlight notice to encourage funding applications that focused on the use of human tissue for 3Rs purposes.

The following awards were made:

- Dr Scott Davies (University of Birmingham), Multiphoton imaging in human liver tissues: validation of a new tool for drug discovery
- Dr Meritxell Huch (University of Cambridge), Replacing liver cancer models by modelling human liver cancer *in vitro*
- Dr Mariya Moosajee (University College London), Generating *in vitro* human optic vesicles to dissect the genetic modifiers affecting ocular maldevelopment

We also hosted two workshops. The first to advance the use of human tissue for oncology research, focused on themes such as improving the use of fresh tissue as an alternative to patient-derived xenograft mouse models, and the second with Safety Pharmacology Society and Coventry University to discuss progress on the implementation of human tissue models for safety assessment.

Previous 3Rs prize winner publishes more ground-breaking research

In 2013, Dr Meritxell Huch from the Gurdon Institute won the 3Rs prize for her work on mouse 'mini-livers'. As a next step to understanding liver disease, her team created 'mini-tumours' from human primary cells. The mini-tumours preserve tissue structure and gene expression patterns of the original tumours they were derived from, making the model biologically accurate.

Dr Huch was also awarded an NC3Rs project grant in 2017 for further validation of the model as an alternative to traditional mouse models.

Broutier L *et al.* (2017). Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. *Nature Medicine* 23: 1424–1435 doi:10.1038/nm.4438.

3Rs resources and training

We provide resources and training to make it easy for scientists at all career stages to engage with the 3Rs. Supporting early career researchers helps to ensure a long-term commitment to the 3Rs.

The Experimental Design Assistant (EDA)

We have continued to support improvements in the design and analysis of animal experiments with our online Experimental Design Assistant. We have focused on promoting the use of the EDA and initiated detailed user testing to improve its functionality. By the end of 2017, there were 3,794 registered users of the EDA.

Assessing the quality of experimental design in grant applications is high on the research funders' list of priorities and part of the armoury for tackling the so-called 'reproducibility crisis'. Much of the information required by funders is based on the work of the NC3Rs. In March, we held a workshop in collaboration with MRC for grant panel members to raise awareness of the importance of good experimental design. Working with funders we have also added an option to the EDA that allows users to download information on their experimental plans in a format that can be imported into grant applications, allowing information to be clearly displayed.

"With the ongoing 'reproducibility crisis' and concerns about the reliability of animal research, it is particularly important to focus on improving experimental design to ensure studies are properly powered and animals are not wasted."

Improving the quality of animal research with the EDA

The EDA was highlighted in two publications in 2017. The first in *Nature Methods* provides an overview of the importance of the role of the EDA and the second in *PLOS Biology* a more detailed description of the EDA functionality, including its tools for randomisation, blinding and selection of statistical test.

Percie du Sert N *et al*. (2017). The Experimental Design Assistant. *Nature Methods*. doi:10.1038/nmeth.4462.

Percie du Sert N *et al*. (2017). The Experimental Design Assistant. *PLOS Biology*. doi:10.1371/journal.pbio.2003779.

The ARRIVE guidelines

In 2010, we published the ARRIVE guidelines, a 20-point checklist designed to improve the reporting of research using animals. Despite the growing support and endorsement from universities, funders and journals worldwide, seven years after their publication, evidence of the impact of the guidelines is limited.

In November 2017, we convened an expert working group with members from the UK, elsewhere in Europe, North America and Australia, to revise and improve the ARRIVE guidelines to maximise their impact. To inform the review, we conducted a survey of researchers to examine how the ARRIVE guidelines are used and to identify reasons for a lack of compliance. Over 300 researchers from 50 countries responded to the survey.

We also interviewed editors from journals with varying levels of endorsement for the ARRIVE guidelines, to understand how journals apply the guidelines and the impact on the editorial workload. To complement this, we co-funded a randomised controlled trial (called the IICARus study) with the BBSRC, MRC and Wellcome, to assess whether a journal (in this case *PLOS ONE*) requesting completion of an ARRIVE checklist improved full compliance with the guidelines.

The findings of the survey and the IICARus study will be available in 2018 and the aim is to publish revised ARRIVE guidelines in 2019.

New resource launched for researchers applying to use animals overseas

To support the peer review service, a new resource was launched in March on choosing contractors for animal research. Aimed at researchers planning to outsource animal work outside of the UK, the resource provides advice on appropriate animal welfare standards.

The new resource has a focus on studies involving dogs and macaques and includes details about required enclosure sizes and space allocations needed for both species, as well as information regarding husbandry requirements. Examples of acceptable and unacceptable housing and husbandry for dogs and macaques are also provided.

New resource for university web pages

University webpages can be a useful tool to highlight how animal research is advancing scientific or medical discoveries and demonstrate commitment to the 3Rs. However, the quality and quantity of content provided on these webpages in terms of the 3Rs is variable. This is a missed opportunity for universities to demonstrate their responsibility and accountability in the use of animals in research.

In May 2017, we published a new guidance document aimed at aiding universities in providing useful and informative animal research webpages. This resource is intended as guided advice for the various stages of preparing these webpages, including their content, design and management.

"The SCHEER Opinion, published in 2017, recognised the NC3Rs role as a catalyst for the advances in 3Rs in NHP research."

Supporting postdoctoral researchers: Fellowships awarded

The NC3Rs Training Fellowship awards, launched in 2016, support promising early career researchers with less than three year's postdoctoral experience, and act as a stepping stone towards an independent research career. Enabling early career researchers to focus on developing new skills and gaining a breadth of research experience relevant to the 3Rs, the scheme aims to nurture future leaders in 3Rs research. Two awards were made in 2017 in addition to two David Sainsbury Fellowship awards, our Fellowship scheme for more established researchers.

2017 was also the first year the annual Fellows' meeting was opened to postdoctoral researchers working on NC3Rs Project Grants. The meeting provided networking opportunities as well as training in skills such as grant writing.

Reviewing our PhD Studentship Scheme

In April 2017 we published a review of our PhD Studentship Scheme highlighting our students and their scientific and 3Rs impacts through a series of case studies. To date we have funded 87 studentships totalling £8.25 million as part of our commitment to embedding the 3Rs in the training and development of graduate scientists.

We hosted our annual summer school for our first-year students in July 2017. The summer school provides an opportunity for students to meet and to learn 3Rs relevant skills. This year it was also attended by 14 students from the Universities of Birmingham, Leicester and Nottingham as part of our regional collaboration with the Midlands universities.

Barriers and solutions for 3Rs progress in NHP research

Whilst progress is being made in applying the 3Rs to NHP research there are a number of barriers that continue to prevent widespread uptake of the available 3Rs techniques. These were highlighted in a publication in *Drug Discovery Today: Disease Models* from Dr Mark Prescott, the NC3Rs lead for NHP welfare activities. The paper, co-authored with Dr Jan Langermans of the Biomedical Primate Research Centre in the Netherlands and Dr Ian Ragan, a member of the NC3Rs Board, also describe potential solutions to each of the barriers such as raising awareness of existing 3Rs techniques and initiatives and improving the provision and quality of staff training.

Prescott MJ *et al.* (2017) Applying the 3Rs to non-human primate research: barriers and solutions. *Drug Discovery Today: Disease Models.* 23: 51-56 doi: 10.1016/j.ddmod.2017.11.001.

Working with partners

Forming effective partnerships with other organisations and sectors is essential to delivery of the NC3Rs mission. In 2017, we continued to focus on building and strengthening collaborations with industry, other research funders and organisations involved in public engagement.

Academia and Funders

NC3Rs-BBSRC Joint Highlight: New approaches to ageing research

Encouraging 3Rs research in areas where there is increasing demand for animal research is critical to the NC3Rs strategy. Our joint research funding highlight notice with the BBSRC on alternative models for ageing research is one example of this. There is an increasing investment in ageing research, both in health and disease, using a range of model organisms, most commonly the mouse. The highlight notice applies to applications received for 2017/18 grant rounds (for the NC3Rs project grants only), with the BBSRC focusing on the healthy aging processes, and the NC3Rs on models of the ageing process relevant to disease. A joint workshop was held in September, with over 60 delegates attending, where speakers gave examples of models and approaches for ageing research from using nematode worms to tissue engineered models to combining *in silico* models with experimentation.

BHF Studentships: Research funding to reduce need for animal studies in cardiovascular research

As part of their commitment to the 3Rs, in 2017, the British Heart Foundation agreed to continue its collaboration with the NC3Rs to fund three PhD Studentships a year to help embed the 3Rs in the training of cardiovascular scientists.

To date seven joint awards have been made including projects developing models of artery injury and aortic aneurysm that minimise the use of animals, exploring the use of the fruit fly as a replacement model, and using tissue-engineered artificial blood vessels to study the molecular mechanisms of blood clotting.

Expanding our peer review service

For over a decade, we have provided a peer review and advice service to UK public funders of bioscience research, primarily for species considered to be of 'special concern', supporting their commitment to high standards of animal research. Over 900 applications involving NHPs, dogs, cats, pigs or equines have been reviewed by our staff, advising funders and applicants on any missed opportunities to implement the 3Rs. The service has also extended to other species, such as rats and mice, where funders have raised concerns about the nature of the procedures or the number of animals requested in the grant application.

In 2017, the peer review service expanded further to include two non-profit partnerships, Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) and Coalition for Epidemic Preparedness Innovations (CEPI). A total of 20 funding bodies are now supported by the peer review service, with applications spanning the breadth of UK biosciences research.

Completed reviews by year

Industry and SMEs

CRACK IT

CRACK IT is the NC3Rs programme that supports collaborations between industry, academia and the SME sector and the commercialisation of 3Rs technologies.

CRACK IT Challenges

CRACK IT Challenges is an R&D competition funded by the NC3Rs to address specific 3Rs challenges that if solved would lead to improved business processes and new marketable products. Challenges are identified in partnership with Sponsors who typically come from the pharmaceutical, agrochemical and consumer product sectors, with the Sponsors providing in-kind contributions to help solve the Challenges. In 2017, there were three Challenges focusing on minimising the use of animals in toxicology studies and improving the utility of *in vitro* methods. The Challenges were sponsored by Shell, Syngenta and Unilever, with the NC3Rs committing over £2.5 million to the competition.

CRACK IT Challenges are run as either single or two phase competitions depending on the level of R&D required.

In 2017, £2M was also awarded to two Newcastle-based SMEs for the second phase of the Osteo-chip and Retina 3D Challenges, with the former being co-funded by the EPSRC and Arthritis Research UK.

CRACK IT Solutions

Through CRACK IT we also provide a technology partnering hub that showcases technologies with 3Rs potential (referred to as CRACK IT Solutions) to help identify new partners to further develop, validate and adopt the technologies. Technologies highlighted on the platform come from SMEs and academic establishments. Where new collaborations are established seed funding is available to facilitate small-scale projects.

In 2017, we increased the funding available from £30k to £50k and made two awards, the first to a project exploring the use of an imaging platform for monitoring corneal wound healing and the second for an *ex vivo* spinal cord slice model for studying pain. Eight new CRACK IT Solutions were showcased through the platform during 2017.

New cell line developed for testing botulinum toxin activity

A team from the University of Sheffield, headed by Professor Bazbek Davletov, published a new cell-based assay for testing the quality and safety of pharmaceutical botulinum neurotoxin serotype B in *Frontiers in Pharmacology*. The most common test for clinical potency is the LD50 bioassay, in mice, which is classified as a severe procedure as it uses death by asphyxiation as an endpoint. The new immunoassay uses an engineered neuroblastoma cell line and has an increased sensitivity compared to the mouse test.

Rust *et al.* (2017). A Cell Line for Detection of Botulinum Neurotoxin Type B. *Frontiers in Pharmacology.* doi.org/10.3389/fphar.2017.00796.

Reviewing the use of a second species in regulatory toxicology studies

Data on the safety of many pharmaceutical compounds is provided by studies in two species typically a rodent (rat or mouse) and a non-rodent (dog, NHP or mini-pig).

During 2017 we continued to work with 37 international companies and regulatory bodies as part of our collaboration with the ABPI to assess whether toxicology data from one species is sufficient to progress a compound through the pharmaceutical pipeline without compromising human safety. We have collected and analysed data on 175 compounds provided by 18 companies and the aim is to publish guidelines on opportunities to reduce the use of two species in regulatory studies in 2018.

Public engagement

We sponsored four events as part of the Pint of Science festival: in Glasgow, London, Oxford and Sheffield, to give our grant holders an opportunity to speak about the 3Rs and their work in an informal setting. The events included two or three speakers who covered a diverse range of subjects from non-mammalian models to computer modelling, tissue engineering and organs-on-chips.

To further support our grant holders, we have raised the amount available through our public engagement scheme to £1,500 as well as increasing the number of times the competition runs each year in order to make the most of the year-round engagement opportunities. In 2017, we made two awards under the scheme, a public lecture with practical demonstrations during an open day at the University of Hull and a lecture and hands-on lab practical for school students at the University of St Andrews.

Annexes

Awards funded through response mode schemes

Project grants

Dr Andrew Broadbent *et al.*,
The Pirbright Institute
A chicken primary B cell culture model to study the pathogenesis and improve the control of immunosuppressive viruses of poultry (£349,071)

Dr Paola Campagnolo *et al.*,
University of Surrey
Ex vivo model for the study of epicardium-targeted therapies (£192,952)

Dr Enrico Dall'Ara *et al.*,
University of Sheffield
A comprehensive *in silico* approach to measure spatio-temporal changes of bone tissue in mouse models of osteoporosis and osteoarthritis. (£357,401)

Dr Ben Davies,
University of Oxford
Reducing the animal cost of CRISPR/Cas9 mutagenesis (£341,783)

Dr Meritxell Huch,
University of Cambridge
Replacing liver cancer models by modeling human liver cancer *in vitro* (£221,496)

Dr Sarah Knowles *et al.*,
Royal Veterinary College
Development, validation and application of enhanced-welfare technology for wild small mammal research (£230,171)

Dr Matthew Leach *et al.*,
Newcastle University
Identifying more clinically effective analgesic regimens and potential strain differences in pain perception in mice using a novel Operant Pain Assay (£298,814)

Dr Alasdair Nisbet *et al.*,
Moredun Research Institute
A technique to test novel methods of controlling poultry red mite in hens without performing field-scale trials (£452,266)

Dr Will Norton *et al.*,
University of Leicester
Quantifying the potential of skin swabbing as a refinement for DNA sampling of laboratory fish (£258,803)

Strategic award

Dr Luigi Margiotta-Casaluci,
Brunel University
Development of an AOP for cardiotoxicity mediated by the blockade of L-type calcium channel (£30,225)

PhD Studentships

Dr Elizabeth Ballou *et al.*,
University of Birmingham
Replacing animal models in fungal pathogen research: An *in vitro* Titan virulence assay for the human fungal pathogen *Cryptococcus neoformans* (£90,000)

Dr Andreas Bender,
University of Cambridge
Moving the Adverse Outcome Pathways Framework towards Practical Utility by Integrating Compound Profiling Data and Using Deep Learning (£90,000)

Dr Karl Butterworth *et al.*,
Queen's University Belfast
Refinement of soft tissue targeting and alignment protocols in small animal radiotherapy using an injectable fiducial marker (£90,000)

Professor Trenton Garner *et al.*,
ZSL Institute of Zoology
Refining, reducing and replacing non-model amphibian experiments on amphibian infectious diseases (£90,000)

Professor Gareth Jenkins *et al.*,
Swansea University
Developing an *in vitro* repeat-dose test as a 3Rs approach to detect non-genotoxic carcinogens (£90,000)

Dr Jan-Ulrich Kreft *et al.*,
University of Birmingham
Developing and validating a computational model of the gut microbiota-mucosa interactions to replace and reduce animal experiments (£90,000)

Professor Paul Langford *et al.*,
Imperial College London
Galleria mellonella - a novel infection model for *Mycobacterium tuberculosis* aimed at reducing the number of animals in experimentation (£90,000)

Professor Anthony Maxwell *et al.*,
John Innes Centre
Insects as models to study the impact of antibiotics and microbiota therapies on the human gut microbiome: reducing the use of animals in research (£90,000)

Dr Mariya Moosajee *et al.*,
University College London
Generating *in vitro* human optic vesicles to dissect the genetic modifiers affecting ocular maldevelopment (£90,000)

Professor Charles Tyler *et al.*,
University of Exeter
An alternative approach for assessing drug-induced seizures, using non-protected larval zebrafish (£90,000)

Dr Simon Waddell *et al.*,
University of Sussex
Characterising *Mycobacterium tuberculosis* in the human lung; developing new tools for antimicrobial drug discovery through the SH-TBL clinical trial (£90,000)

PhD Studentships with joint BHF Funding

Professor Paul Evans *et al.*,
University of Sheffield
Using zebrafish embryos to identify genes that protect against atherosclerosis (£90,000)

Dr Alan Harper *et al.*,
Keele University
Recreating thrombosis models using tissue-engineered arterial constructs: A novel method to reduce and replace mice used in platelet research (£90,000)

Dr Stephen White *et al.*,
Manchester Metropolitan University
Development of E-Sense: a flexible *in vitro* platform to determine cardiovascular risk (£90,000)

David Sainsbury Fellowships

Dr Olga Baron,
King's College London
Drosophila model for muscular pain (£268,127)

Dr Anne Herrmann,
University of Liverpool
Development of the chick embryo as a replacement for rodent models of tumour metastasis (£239,586)

Training Fellowships

Mr Scott Davies,
University of Birmingham
Multiphoton imaging in human liver tissues: validation of a new tool for drug discovery (£109,410)

Dr Carola Morell,
University of Cambridge
Novel approach to model Non-Alcoholic Fatty Liver Disease using human Pluripotent Stem Cells (£115,646)

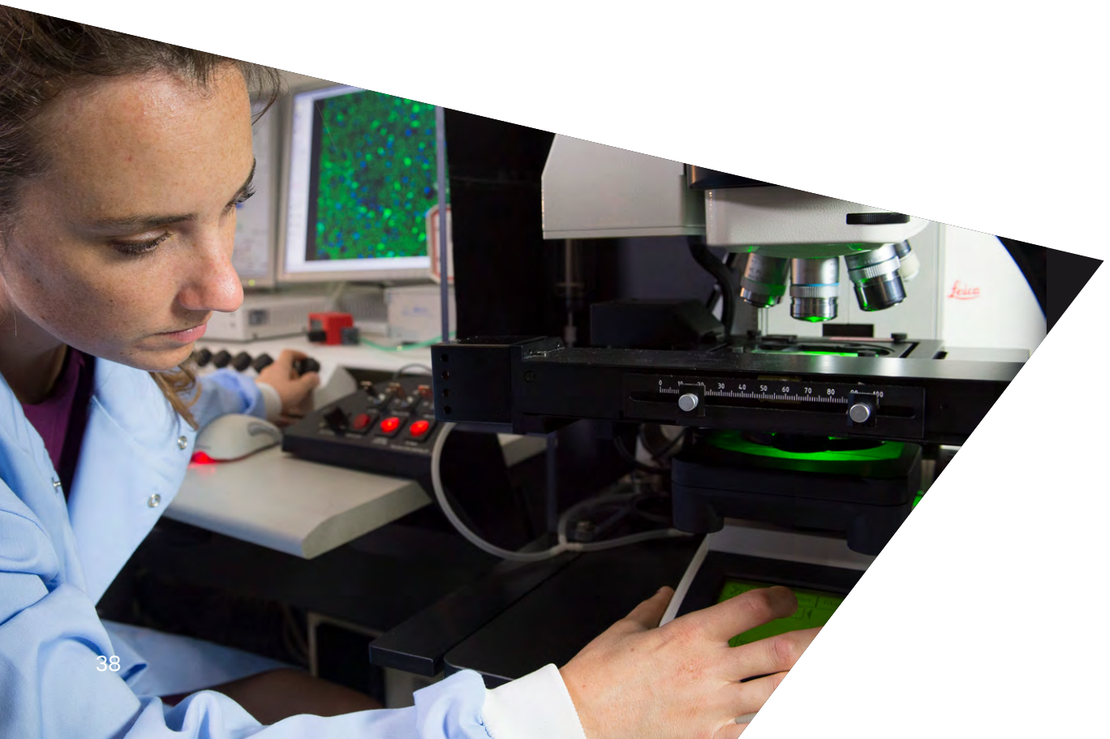
Skills and Knowledge Transfer grants

Dr Raymond Bujdoso *et al.*,
University of Cambridge
The use of PrP transgenic *Drosophila* to replace and reduce mice in the bioassay of mammalian prions (£75,608)

Professor Joanne Cable,
Cardiff University
High-throughput *in vitro* culture system for Cryptosporidium oocysts: replacing animals in research (£75,608)

Dr Claire Gibson,
University of Leicester
Dissemination of refinements in mouse experimental stroke models to the scientific community (£65,220)

Dr Rachel Tanner *et al.*,
University of Oxford
Transfer of a non-human primate (NHP) *in vitro* functional assay for the early evaluation of TB vaccine candidates and the associated immune response (£75,608)



Summary of award rates by scheme

Funding scheme	Number of applications received	Number of awards	Award rate (%)
Project grants – full applications	24*	9	38%
Strategic awards	3	1	33%
PhD Studentships	61	15**	25%
David Sainsbury Fellowships	10	2	20%
Training Fellowships	17	2	12%
Skills and Knowledge grants	11	4	36%

*Proceeded by a formal outline stage to which 89 applications were received and 26 were invited to submit a full application.
**One PhD Studentship award was withdrawn.

Awards funded through CRACK IT Challenges

2016 CRACK IT Challenges: Phase 2 awards

Osteo-chip

Sponsors: GlaxoSmithKline. The EPSRC and Arthritis Research UK also provided co-funding to support this Challenge.
Contractor: Professor Anne Dickinson, Alcyomics Ltd (£999,996)

Retinal 3D

Sponsors: Merck, Novartis and Roche
Contractor: Professor Lyle Armstrong, Newcells Biotech Ltd (£1,000,000)

2017 CRACK IT Challenges: Phase 1 awards

DARTparths

Sponsors: Shell and Syngenta
Contractor: Dr Paul Andrews, SimOmics Ltd (£99,606), Dr Barry Hardy, Douglas Connect GmbH, (£99,834), Dr Raymond Pieters, University of Applied Sciences Utrecht (UASU)/ Institute for Risk (£100,000)

DoCE

Sponsors: Shell and Unilever
Contractor: Dr Tanja Hansen, Fraunhofer Institute for Toxicology and Experimental Medicine, (£99,371), Dr Luise Henneberger, Helmholtz Centre for Environmental Research (£93,194), Professor Philipp Mayer, Technical University of Denmark (£99,790)

2017 CRACK IT Challenges: Single Phase award

RespiraTox

Sponsors: Shell
Contractor: Dr Sylvia Escher, Fraunhofer Institute for Toxicology and Experimental Medicine (£99,996)

CRACK IT Solutions

Dr Will Krawszik, Moleculomics Ltd
£50,000
Cross-species comparative *in silico* platform (CRISP)

Dr Marloes Peeters, Manchester Metropolitan University
£49,011
A new sensor platform combining 'plastic' antibodies with simple thermal detection

Events organised by the NC3Rs in 2017

Applying exposure science to increase the utility of nonanimal data in efficacy and safety testing

15-16 February, London

A workshop held jointly with Unilever to bring together academic and industry researchers across multiple disciplines to share their knowledge and experiences in applying exposure science to increase the utility of *in vitro* and *in silico* data for decision-making, efficacy and safety assessment.

Human tissue models for cancer research

01-02 March, London

A workshop to bring together cancer researchers working with human tissue and other models to discuss how human tissue is used and what barriers exist to increased uptake.

Systematic reviews in animal research: Launch of SyRF

30 March, London

The NC3Rs, in collaboration with the CAMARADES group, hosted a half-day seminar on systematic reviews in animal research. At this meeting we launched the CAMARADES-NC3Rs Systematic Review Facility (SyRF) web app – a new free-to-use tool to help researchers perform systematic reviews and meta-analyses of animal studies.

Improving peer review of *in vivo* research proposals

31 March, London

A workshop, held jointly with the MRC, to support panel members to assess the quality of the planned experimental design in grant proposals.

NC3Rs Fellows Meeting

27-28 April, London

Annual meeting of NC3Rs Fellows and NC3Rs funded postdoctoral researchers to provide training and development opportunities in the 3Rs as well as skills such as grant writing.

NHP NACWO Network meeting

21 April, Newcastle

Annual meeting of NACWOs to promote sharing of experience and practical information about NHP care and use and to identify opportunities for refinement and the steps needed to achieve this.

The use of human tissues for safety assessment

03 May, Coventry

A workshop, co-organised by the NC3Rs, SPS and Coventry University to bring together researchers from academia, SMEs and the pharmaceutical industry to discuss how best to support and enable the use of human tissues in safety testing.

NC3Rs Student Summer School

19-21 July, Nottingham

Annual meeting of NC3Rs students and other students with an interest in the 3Rs. As well as increasing understanding of the 3Rs, the Summer School aims to provide the students with an opportunity to learn about best practice in experimental design, PhD management strategies, public engagement, and future career paths following a science PhD.

Towards global elimination of the acute toxicity 'six-pack'

20 August, Seattle

A workshop in collaboration with the US National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the European Commission's Joint Research Centre (JRC). The focus of the workshop was on current and future efforts to eliminate requirements for the acute toxicity 'six-pack' of *in vivo* studies (acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, acute eye irritation, acute dermal irritation, and skin sensitisation).

Workshop on Ageing Research

01 September, London

A workshop, in collaboration with BBSRC, to outline a joint funding highlight notice to encourage the development of new and innovative models and approaches for ageing research, which reduce the current reliance on mammalian models, as well as address the gaps in terms of scientific utility and relevance to human ageing.

Mouse Handling Workshop: Support for trainers, NTCOs and other named persons

27 September, London

A workshop to support establishments to move to the refined techniques of tunnel and cup handling to avoid or reduce negative responses in mice, leading to more robust and reliable responses in behavioural tests, benefiting the science the animals are used for.

Workshop on Amphibian Welfare

05 October, London

A workshop in collaboration with the Zoological Society of London, on amphibian welfare. This brought together experts from the laboratory, zoo and field communities to explore approaches that can be taken to help improve conditions for amphibians in research, and take initial steps in establishing common principles for their housing and care.

Primate Welfare Meeting

03 November, London

Annual meeting for laboratory personnel working directly with non-human primates to share best practice in the use and care of non-human primates internationally.

NC3Rs Board members

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University of Southampton

Dr Paul Brooker
Independent

Professor Chris Denning
University of Nottingham

Dr Paul Finnemore
AstraZeneca

Professor Paul Garside
University of Glasgow

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Regional Programme Manager (Midlands)

Dr Cathy Vickers
Head of Innovation

Courtney Williams
Online Communications Officer

Acronyms

3Rs
Replacement, reduction and refinement of animals in research

ABPI
Association of the British Pharmaceutical Industry

AOP
Adverse Outcome Pathway

ARRIVE
Animal Research: Reporting of *In Vivo* Experiments

BBSRC
Biotechnology and Biological Sciences Research Council

BHF
British Heart Foundation

CAMARADES
Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies

EDA
Experimental Design Assistant

EPSRC
Engineering and Physical Sciences Research Council

FCP
Fixed Concentration Procedure

IAT
Institute of Animal Technology

IMPROVE
Ischaemia Models: Procedural Refinements Of *in Vivo* Experiments

MHRA
Medicines and Healthcare products Regulatory Agency

MRC
Medical Research Council

NACWO
Named Animal Care and Welfare Officer(s)

NHP
Non-human primate

OECD
The Organisation for Economic Co-operation and Development

SCHEER
Scientific Committee on Health, Environmental and Emerging Risks (SCHEER)

SME
Small and Medium-sized Enterprise

SPS
Safety Pharmacology Society

SyRF
Systematic Review Facility

ZSL
Zoological Society of London

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