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

Workshop report

Applying the 3Rs in pharma: Improving delivery of innovative medicines to patients

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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.

The Toxicology and Regulatory Sciences team

The Toxicology and Regulatory Sciences team consists of three Programme Managers (Dr Natalie Burden, Dr Helen Prior and Dr Fiona Sewell) supported by two Science Managers (Nikki Gellatly and Dr Briony Labram). Our large programme of work covers both human and environmental health and embraces many industry sectors, including pharmaceuticals, agrochemicals and industrial chemicals.

Funding from the Association of British Pharmaceutical Industry (ABPI) has ensured dedicated support for advancing the 3Rs in pharmaceutical research and testing, currently focussed on supporting the position held by Helen Prior. Specific projects are also managed by other NC3Rs Programme Managers, in particular Fiona Sewell, and Dr Samuel Jackson (who leads on safety pharmacology topics).

The team is also supported by Professor Ian Kimber, the NC3Rs Toxicology Ambassador. In this role, he acts as an international ambassador for our Toxicology and Regulatory Sciences programme to foster our connections with other global 3Rs initiatives, particularly in the USA.

Over the past 15 years, the NC3Rs has developed a strong and effective collaboration with the pharmaceutical industry. We have worked closely with pharmaceutical and biotechnology companies, contract research organisations (CROs), regulatory bodies and academia from the UK and elsewhere in Europe, the USA and wider international community to review best practices and identify important opportunities to consider and develop modified or new approaches to safety assessment in drug development.

The NC3Rs supporting the pharmaceutical industry

A summary of how the NC3Rs works to support the pharmaceutical industry is provided below:

Sharing data and experience

As an independent scientific organisation the NC3Rs is uniquely placed to act as an 'honest broker', providing a platform to share and anonymise pre-competitive data and case-studies. This approach has been at the heart of many of our projects and we have shared data on over 1,000 compounds from 111 different companies within expert working groups on 17 topics since 2004. Some of these were discussed during the meeting within presentations or posters (see Annexes 1 and 2), whilst other projects include the appropriate use of non-human primates (NHPs) for mAb development, reducing the use of recovery animals and use of human tissue for safety assessment. Such collaborations have created evidence bases that could not be achieved by any one organisation alone, providing the impetus for changes in company practice and, in some cases, international regulations – for example, the removal of acute toxicity studies from the ICHM3 guideline (ICHM3(R2), 2009) and the release of Q&A guidance for incorporation of microsampling for toxicokinetic assessments (ICHS3 Q&A, 2017). We also host scientific events and symposia and provide online resources and regular newsletters to disseminate information about our work nationally and internationally.

CRACK IT

CRACK IT is our open innovation programme, which connects industry, academia and small and medium enterprises (SMEs) to support the development and application of marketable products with 3Rs and scientific benefits.

The programme consists of two schemes:

CRACK IT Challenges: An annual competition that funds collaborations between industry, academics and SMEs to solve scientific and business challenges identified by the bioscience sector. Since 2011, the NC3Rs has launched over 30 CRACK IT Challenges, 20 of which were proposed and supported with in-kind contributions from 16 pharmaceutical sponsors. We have invested almost £19 million in 3Rs technology which directly benefit the pharmaceutical industry. Five of the projects were discussed during the meeting in poster presentations (see Annex 2).

CRACK IT Solutions: A technology partnering hub for academics and SMEs to showcase their 3Rs technologies to the wider scientific community, to identify new partners to adopt, develop and validate the technology to maximise uptake. The NC3Rs has invested over £700,000 in the programme since 2012 and leveraged a further £300,000 from external contributions, to fund 20 projects with collaborations involving 19 pharmaceutical or biotechnology companies.

We have recently introduced a new Innovation Platform which better showcases our programmes, their progress and outputs, making it easier for you to get involved. We have also published a CRACK IT Review which reflects on the first eight years of CRACK IT and how it has achieved business, scientific and 3Rs impacts through open innovation and collaboration.

Funding for basic research and early career development

Funding research is a key part of the NC3Rs strategy to provide robust and reliable 3Rs models, technologies and tools that enhance scientific discovery. Since the NC3Rs was launched in 2004, we have committed £65 million in grants and early career awards to advance the 3Rs, with 332 awards at 80 research institutions, funding more than 560 principal and co-investigators and supporting the development of 144 PhD students and fellows. Many of the awards address topics relevant to the pharmaceutical industry, including cancer, stroke, infectious diseases and cardiotoxicity. Recently, we have launched a strategic collaboration with Medicines Discovery Catapult for a new Technologies to Tools (T2T) scheme. This will support the translation of *in vitro* models and non-animal technologies, developed with NC3Rs grant funding, into research-ready products and services that can be applied effectively in the pharmaceutical industry.

Promoting high standards in the use and welfare of laboratory animals

Our focus on the link between good animal welfare and the quality of research data covers many aspects relevant to industry. These include the appropriate design and reporting of animal research (the Experimental Design Assistant and ARRIVE guidelines), refinement of mouse handling, adoption of social-housing for telemetry studies, implementation of microsampling and our NHP programme and annual primate welfare meeting.

Background

Over the last decade, a major focus of innovation in the pharmaceutical industry has been the development of human-relevant *in vitro* models, including microphysiological systems. Additionally, *in silico* modelling and artificial intelligence (AI) are increasingly used to explore the potential for existing datasets to predict adverse effects and characterise toxicity profiles of new medicines in drug discovery and development. These changes are partly a reflection of the acknowledged problem of attrition and low productivity – whereby only five to ten per cent of development projects that enter clinical trials result in a new medicine. One important reason for this is the lack of translation from experimental animal data to human volunteer and patient efficacy and safety. There has also been a rapid evolution of new treatment modalities, moving away from small molecule or monoclonal antibody platforms to include, for example, modified mRNA, antisense oligonucleotides and chimeric antigen receptor T cell (CAR-T) therapies. These have challenged conventional thinking in terms of the *in vitro* and *in vivo* models required, and in some cases, very little or no *in vivo* toxicity testing is considered relevant prior to clinical trials or marketing approval. These new modalities are often intended for serious or life-threatening conditions, and much of the safety testing is performed during the clinical trials. Whether these principles can be extended to medicines for less serious conditions, or for other modalities, remains an important and intriguing question.

Enhancement of the established animal toxicology packages with more human-relevant *in vitro* data and *in silico* simulations aligns closely with the principle of 3Rs and has the potential to replace, refine or reduce animal use in the future.

The NC3Rs is in a unique position as an independent scientific organisation to support and manage collaborations between industry and regulators where non-animal approaches are used and whilst the requirement for safety (toxicology) testing in animals remains. Through sharing ideas and best-practice case-examples, opportunities can be identified for application of the 3Rs and to support accelerated delivery of new medicines to patients. This may include identifying tests that are redundant (tests performed but the data not used, or where relevant information is available from other studies), reduction of animal numbers through use of optimised study designs, or refinements in procedures or housing to improve animal welfare and data obtained.

The lack of global harmonisation of animal toxicity testing within regulatory requirements is a major hurdle for application of the 3Rs. Companies are generally aiming for registration in as many geographic regions as possible and therefore data packages often reflect the needs (or perceived expectations) of the regulatory authority that requires the most data. That is, some studies may be performed for submission to one region that are not required by others. With the aim of global harmonisation in mind, the work of the NC3Rs is becoming increasingly international, with growing input from the global community to improve implementation of 3Rs recommendations worldwide.

The workshop

In June 2019, the NC3Rs hosted a workshop in London to highlight our approaches to working with and supporting the pharmaceutical industry. The current challenges faced by the industry were defined and discussed, to frame the future direction of the NC3Rs pharmaceutical industry-driven programme, including potential new projects.

The event brought together 62 scientists from six academic groups, nine CROs, 21 pharmaceutical companies, five regulatory/governmental bodies and five SMEs from across the UK, elsewhere in Europe and the USA. A copy of the event programme can be found in Annex 1. The presentations covered:

- The role and impact of data-sharing activities and perspectives from industry, CROs and regulators on benefits and challenges of participating in NC3Rs working groups.
- Projects with 3Rs relevance from three external consortia:
  1. 'Optimal duration of non-clinical studies to assess the safety of monoclonal antibodies.' *European Partnership for Alternative Approaches to Animal Testing and the Medicines Evaluation Board.*
  2. 'Predicting the safety of medicines in pregnancy: A new era?' *Medicines and Healthcare products Regulatory Agency (MHRA).*
  3. 'Animal-free development of Advanced Therapy Medicinal Products (ATMPs): Is it possible?' *Medicines Evaluation Board.*
- Three 'hot topic' areas – biosimilars, longer-term toxicity studies (i.e. 13-39-week duration) and juvenile animal toxicity studies – which were then the focus for breakout sessions discussions.

Opening and closing keynote presentations were given by Dr Chris Powell (GlaxoSmithKline) and by Dr Stefan Platz (AstraZeneca), covering how pharmaceutical innovation aligns to the 3Rs and the challenges of safety assessment for new therapeutics, respectively. These presentations outlined the current status of the pharmaceutical industry and opportunities to reduce attrition via use of more predictive non-animal approaches. Posters were presented by NC3Rs-funded researchers, CRACK IT Challenge project teams, NC3Rs Programme Managers and representatives of other industry consortia, highlighting the breadth and depth of ongoing or potential 3Rs projects relevant to pharmaceutical toxicology (see Annex 2).

This report summarises presentations and discussions from each of the breakout sessions, and the 3Rs challenge areas identified by delegates. This will inform future NC3Rs activities for the continued promotion and application of the 3Rs in pharmaceutical safety assessment.

Harmonisation of *in vivo* requirements for biosimilar mAbs

Biosimilars are biological medicinal products that contain a version of the active substance of an already authorised original biological medicinal product (the innovator or reference product). Testing relies on demonstrating the biosimilar is highly similar to the reference product in terms of quality characteristics and biological activity. The purpose of this is to ensure that the previously established safety and efficacy profile of the reference product also applies to the biosimilar.

There are currently regional differences in biosimilar regulations, leading to variability in the *in vivo* nonclinical toxicity testing performed to support clinical development and marketing of biosimilars. The European Medicines Agency (EMA) guidance (2014) describes an approach that enables biosimilars to enter clinical trials based on robust *in vitro* data alone, whilst the US Food and Drug Administration (FDA) guidance (2015) is ambiguous, implying there may be appropriate scientific justifications 'for not conducting an animal toxicity study' and that early discussions with the agency are encouraged. However, in other regions it is less clear whether *in vitro* only data packages are sufficient, for example, the World Health Organization (WHO) guidance (2016) is followed in many emerging markets and despite recent updates, is generally interpreted to mean *in vivo* toxicity studies are required.

In 2014 the NC3Rs, together with the MHRA, convened a large international working group to evaluate and address the challenges in this area. Although the working group recommended waiving of *in vivo* studies (Chapman *et al.,* 2016), opportunities to use *in vitro* only packages are limited as differences in regional requirements still remain. Indeed, for the new biosimilars approved by the EMA and FDA up to 2018, all packages contained some animal data (Pipalava *et al,.* 2019). During the workshop, a retrospective review of biosimilars within the Pfizer portfolio was presented and concluded that determination of similarity could have been made without conducting *in vivo* studies, in agreement with the NC3Rs working group findings.

The breakout participants were not aware of any examples where *in vitro* data alone has been accepted for biosimilar mAbs, although experience within the group indicated that the EMA, FDA and Health Canada may be amenable to this approach in the near future. Other regions including China, India, Japan, Korea and Russia continue to expect and request *in vivo* studies, therefore companies are likely to perform *in vivo* studies to allow global marketing of their products. The group shared examples of minimal study designs (e.g., one sex, one dose group) and use of rodents rather than non-rodents that are currently being accepted. However, no one said that they would be confident to submit *in vitro* only data packages without prior discussion with regulators, especially if the *in vivo* data may already be available for submission to other regions.

It was agreed that cross-company collaboration is required to publicise case studies of the more minimal *in vivo* approaches and examples where *in vivo* studies do not add value in assessing biosimilars, to increase confidence and work towards global regulatory acceptance of *in vitro* only data packages.

Longer term* toxicity studies

A recent NC3Rs/ABPI collaboration reviewed how and when two species are used within pharmaceutical regulatory toxicology studies, and whether a rodent and a non-rodent species are still required in the current industry landscape (Prior *et al,.* 2018). This project initially focused on whether existing opportunities to use a single species are being fully exploited and/or could be expanded. Within the current paradigm for the development of biologicals, reduction to a single species for longer-term studies may be possible if similar target organ toxicity profiles are identified in two species within the short-term studies, as outlined within the ICHS6(R1) guideline (ICHS6(R1), 2011). Preliminary evidence supports this for biologics and potentially other modalities (e.g. small molecules). However, applying these opportunities to the current nonclinical toxicology strategies for other molecule types and incorporation into the regulatory guidelines would need more robust evidence to demonstrate minimal impact on human risk assessment.

The critical information required to justify the decision to reduce to a single species is the acceptance of 'similar toxicities from short-term studies'. A harmonised definition or sharing of case examples for 'how' companies have interpreted the guidelines to successfully justify the use of one species, plus an understanding of the barriers that currently restrict wider adoption, may be useful.

There are different approaches among companies in terms of timing of regulatory interactions. For example, some seek advanced regulatory approval for the decision to start longer-term studies in one species, whereas others make the decision and progress without seeking regulatory advice. Delegates reported that some regulatory authorities may request data from two species as a conservative (data-rich) approach and that this also reduces risk when aiming for global regulatory acceptance. It would therefore be useful to know more about the different interactions. For example, how often have companies progressed with one species with no subsequent requests for additional studies? Is this as a result of feedback with regulators in advance, and/or due to regional variability in acceptance of this approach?

Further work is required to build a more substantive evidence base to define the specific circumstances it may (or may not) be appropriate to progress in a single species for longer-term studies. Limitations of the current data set includes increasing the number of molecules using two species for individual modalities and provision of more detailed information on target organ severity, exposure and other data within the package. A further data set focused towards molecules with both short- and longer-term studies could provide cross-industry experience to expand on previous work, investigating the incidence of new nonclinical toxicities upon longer-term dosing (Galijatovic-Idrizbegovic *et al*., 2016; Roberts *et al*., 2015). This may quantify the risk of potentially 'missing' toxicities that impact clinical development if only a single species were used and would be important to identify human-relevance for particular therapy areas or molecule-types.

Future replacement of the data generated in one of the nonclinical species with *in vitro* or *in silico* human-relevant data or by leveraging AI/machine learning opportunities may also be possible.

* longer-term refers to the studies generally conducted to support Phase II/III clinical trials of 13-, 26- or 39-week duration.

Juvenile animal toxicity studies

The juvenile animal study (JAS) is a key component of the nonclinical safety assessment of paediatric drugs. A new draft ICH guideline (ICHS11, 2018) recommends international standards and promotes harmonisation of the nonclinical safety studies suporting the development of paediatric medicines, whilst advocating 3Rs principles. Weight of Evidence (WoE) factors, such as the youngest intended patient age and whether there are known (or suspected) adverse effects on developing organ systems of patients during the intended paediatric trial, contribute to determining if nonclinical studies are warranted. The JAS study design should contain core endpoints (mortality and clinical observations, growth, food consumptions, sexual development, anatomic pathology and toxicokinetics) with optional endpoints (e.g., ophthalmology, central nervous system (CNS) and reproductive assessments) that are only added to address specific potential safety concerns. Whilst study designs may vary significantly, these are large and complex studies, with animal usage exceeding any other study in the nonclinical programme. It is vital therefore that all options to reduce and refine these studies are explored.

Although a high number of JAS are being performed, it is unclear how many of the studies are required or how useful they are to enable safe administration of drugs in a paediatric population (Baldrick, 2018). For example, to what extent are new toxicities or safety concerns identified in juvenile animals, and would they be predictable from exposure/maturation differences? Discussions centred around two aspects 1) investigating the value/relevance of the studies (i.e. opportunities to reduce the number of studies required) and 2) how to refine studies that are performed.

Better understanding of current approaches and the value of JAS within toxicology packages is needed. This includes whether effects identified are relevant, and whether the data is used in decision making and genuinely impacts labelling; or if these studies are simply a "comfort factor" for trials in children? The value of individual components was discussed, in particular the CNS assessments – how often are there different effects *versus* adult animal data (e.g., in CNS safety pharmacology studies)? The group was unaware of any alternative methods for paediatric toxicity, and the ability for pharmacokinetic/pharmacodynamic (PK/PD) models to be developed from adult data was discussed. Incorporation of in silico techniques could add to the WoE decision to determine whether a JAS is warranted, and could thus reduce the number of studies in the future.

Selecting animals for study can have significant impact on total animal usage and the robustness of data. One pup selection method commonly used (the 'between litter allocation' method) only allocates a small number of the dam's natural offspring per litter. Malcolm Blackwell (Sequani) presented the 'cross-fostering' approach, whereby offspring are distributed to multiple litters, removing any genetic bias or culling, and maximising offspring use. This reduces overall animal usage (typically by >65%), provides better quality data and greater study flexibility. Delegates felt the 'cross-fostering' approach is widely applicable, since there are limited numbers of breeders/suppliers and extensive experience from at least two UK CROs, who would be willing to promote this 3Rs opportunity further. Sequani have demonstrated a reduction of approximately 20,000 rats over 10 years which could have a considerable impact if adopted by other CROs worldwide. Collaboration and publication of this approach is important to raise awareness and to overcome associated challenges, such as supplier availability and experience compared to the 'between litter' approach, and various aspects around practical feasibility.

Summary of the current 3Rs challenge areas

Delegates were given the opportunity to provide insight on current 3Rs challenge areas relevant to their expertise or generally within the topic of pharmaceutical toxicity testing. These were intended to help inform our strategy for future NC3Rs activities and fell broadly within three themes (see Annex 3 for the full list):

1. Alternative systems/methods – replacement of animal toxicity studies. Examples included 'Could we develop a PK/PD model that could predict new exposures and assess if *in vivo* bridging work is actually needed (e.g. for repurposing, patent extensions, new dose route, new indication etc.)'; 'Organ-on-a-chip systems (or combination of models): are they fit for purpose yet? Would a test set of cross-pharma compounds help validate models?'

2. Toxicology assessments and study designs – minimising animal use and refining procedures when animals continue to be required for regulatory toxicology assessments. Examples included 'Are spare animals really needed to be ordered as often as they are?'; 'Explore the value of urinalysis – value of the data (is it used?) *versus* individual housing and procedures involved'; 'Can we reduce the number of animals on non-rodent chronic studies – do we really need 4M+4F?'

3. Reviewing global practice – influencing regulatory change and global harmonisation. Examples included: 'China still expect acute toxicity studies for pharmaceuticals, despite removal of requirements from ICHM3, this is the only region where these tests may be required?'; and 'Can we stop doing nine-month non-rodent studies and do six-month studies only instead?'

Concluding remarks

It is clear that there are still many opportunities to replace, refine or reduce the use of animals in pharmaceutical safety evaluation and that the industry remains dedicated to identifying new ways of working that minimises the use of animals without compromising the safety of medicines. The increase in innovative treatments and new drug modalities has challenged the design of conventional toxicology programmes, often requiring little nonclinical data in support of human trials. If the industry continues to evolve and expand in this way, there may be a natural decline in the animal toxicity studies performed. The rapid advancement and development of new technologies, in conjunction with *in vitro* and *in silico* methodologies, also aligns well with the 3Rs. More human-relevant data can be provided earlier within the drug development pipeline, often replacing animal screening and/or promoting candidates with fewer adverse effects in subsequent animal tests. These new methods may ultimately contribute towards a decrease in drug attrition and increase the availability of new medicines to patients.

To continue to reduce the reliance on animal toxicity tests, further non-animal methods that are more predictive than traditional methods and improve safety assessment are required. A number of the NC3Rs programmes investigate 'replacement' opportunities, such as funded research, CRACK IT projects and collaborations with other industry partners, for example with the Medicines Discovery Catapult for organ-on-a-chip technologies. The Toxicology and Regulatory Sciences team has also led a programme to encourage the development and application of adverse outcome pathways (AOPs) which harness the predictive potential of non-animal methodologies; our programme included strategic funding for the development of AOPs applicable to cardiotoxicity.

Other themes identified during the workshop were to expand on previous work (e.g., biosimilars, microsampling) and the role of the NC3Rs for new topics, such as JAS. A number of the 'toxicology assessments and study design' ideas will be addressed together within a future workshop. The NC3Rs will continue to build collaborations with other consortia and organisations to progress projects to promote broad participation and wider international impact of 3Rs opportunities. We value our relationships and collaborations with European and US organisations and regulators such as the European Commission, Joint Research Centre (JRC), EMA, FDA, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and National Toxicology Program (NTP) and aspire to build on our activities with these groups. We acknowledge the need to foster new relationships with companies and regulators within other regions (for example China, Japan, India and South America) to maximise the full 3Rs potential of project recommendations.

Acknowledgements

The NC3Rs is grateful to the speakers and poster presenters for their contributions to the workshop and to all current and past members of our working groups.

Representatives from the following organisations participated in the workshop discussions:

- Association of British Pharmaceutical Industry (UK)
- ApconiX (UK)
- Aptuit (Evotec, Italy)
- AstraZeneca (UK)
- Bayer Pharma (Germany)
- Biomedical Primate Research Centre (The Netherlands)
- Brunel University London (UK)
- Certara (UK)
- Charles River Laboratories (UK and The Netherlands)
- Citoxlab (France)
- Clyde Biosciences (UK)
- Covance (UK)
- Eli Lilly and Company (USA)
- Finnish Medicines Agency (Finland)
- Genentech (USA)
- Gilead Sciences (USA)
- GlaxoSmithKline (UK)
- iBiologix (UK)
- IMOL Preclinical services (UK)
- Janssen (Belgium)
- Medicines Evaluation Board (The Netherlands)
- Merck (UK)
- Medicines and Healthcare products Regulatory Agency (UK)
- Mimetas (The Netherlands)
- MundiPharma (UK)
- Newcells Biotech (UK)
- Novo Nordisk (Denmark)
- Orchard Therapeutics (UK)
- Pfizer (USA)
- Sequani (UK)
- Sanofi (France)
- Sosei Heptares (UK)
- UCB (UK)
- Home Office (UK)
- University of Manchester (UK)
- University of Oxford (UK)
- VAST Pharma Solutions (UK)

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Glossary of terms

ABPI	Association of British Pharmaceutical Industry
ARRIVE	Animal Research: Reporting *In vivo* Experiments
ATMPs	Advanced therapy medicinal products
CNS	Central nervous system
CRO	Contract research organisation
EMA	European Medicines Agency
EPAA	European Partnership for Alternative Approaches to Animal Testing
ePPND	Enhanced pre- and post-natal development
FDA	US Food and Drug Administration
GLP	Good Laboratory Practice
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
JRC	Joint Research Centre (European Commission)
mAbs	Monoclonal antibodies
MHRA	Medicines and Healthcare products Regulatory Agency
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NHPs	Non-human primates
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NTP	National Toxicology Program
PMDA	Japanese Pharmaceuticals and Medical Devices Agency
PK/PD	Pharmacokinetic/Pharmacodynamic
SMEs	Small and medium enterprises
WHO	World Health Organization

Annex 1: Event programme

10:30-11:00	**ARRIVAL and REFRESHMENTS**
11:00-11:20	**Welcome and introduction to interactions with the NC3Rs** *Professor Ian Kimber OBE, University of Manchester (Chair)*
11:20-11:50	**Opening keynote: Pharmaceutical Innovation – Alignment with the 3Rs** *Dr Chris Powell, GlaxoSmithKline*
DATA-SHARING WORKING GROUPS	
11:50-12:10	**Role and impact of data-sharing working groups** *Dr Fiona Sewell, NC3Rs*
12:10-12:25	**An industry perspective of the impact of NC3Rs working group benefits and challenges** *Dr Leigh Ann Burns Naas, Gilead*
12:25-12:40	**A CRO perspective of NC3Rs working group benefits and challenges** *Dr Ankie Schoenmakers, Charles River*
12:40-12:55	**A regulatory perspective of NC3Rs working group benefits and challenges** *Dr David Jones, Medicines and Healthcare products Regulatory Agency*
12:55-14:10	**LUNCH and POSTER VIEWING**
HIGHLIGHTING OTHER 3Rs CONSORTIA	
14:10-14:20	**Optimal duration of non-clinical studies to assess the safety of monoclonal antibodies** *Dr Lolke de Haan, AstraZeneca and Dr Peter van Meer, Medicines Evaluation Board*
14:20-14:30	**Predicting the safety of medicines in pregnancy: A new era?** *Dr Ross Hawkes, Medicines and Healthcare products Regulatory Agency*
14:30-14:40	**Animal-free development of Advanced Therapy Medicinal Products (ATMPs): Is it possible?** *Dr Jan Willem van der Laan, Medicines Evaluation Board*
SCOPING FOR FUTURE 3Rs OPPORTUNITIES	
14:40-14:50	**Are animal studies necessary for development of biosimilars?** *Dr Michael W Leach, Pfizer*
14:50-15:00	**Opportunities for wider use of a single species for longer-term toxicology studies** *Dr Helen Prior, NC3Rs*
15:00-15:10	**Juvenile animal toxicology studies: 3Rs opportunities** *Dr Malcolm Blackwell, Sequani*

Annex 1: Event programme

BREAKOUT SESSIONS with refreshments	
15:10-15:20	**Introduction to the breakout sessions**
15:20-16:00	**Breakout sessions** **1. Biosimilars:** *Michael W Leach, Pfizer and Fiona Sewell, NC3Rs* **2. Longer-term toxicity studies:** *David Clarke, Lilly and Helen Prior, NC3Rs* **3. Juvenile toxicity studies:** *Malcolm Blackwell and Natalie Burden, NC3Rs*
16:00-16:30	**Feedback from breakout sessions** *Rapporteurs*
16:30-16:50	**Open discussion on further ideas for future 3Rs opportunities** *Professor Ian Kimber OBE, University of Manchester (Chair)*
16:50-17:20	**Closing keynote: The challenges of safety assessment for new therapeutics** *Dr Stefan Platz, AstraZeneca*
17:20-17:30	**Wrap up and close of meeting** *Professor Ian Kimber OBE, University of Manchester (Chair)*
NETWORKING RECEPTION	
17:30-18:30	**Networking reception and poster viewing**
18:30-20:00	**Hot buffet sit-down dinner**

Annex 2: Poster presentations

NC3Rs office-led programmes

- Results of a survey on the use of the functional observational battery (FOB) and Irwin tests in central nervous system (CNS) safety pharmacology. *Dr Sam Jackson, NC3Rs*
- Can we expand the use of one species for post-'First-in-Human' (FIH) studies, within and beyond ICHS6? *Dr Helen Prior, NC3Rs*
- Social housing during telemetry studies in rodents and non-rodents. *Dr Helen Prior, NC3Rs*
- Trends toward a reduction in the use of recovery animals for first-in-human (FIH) studies from 2013 to 2017. *Dr Fiona Sewell, NC3Rs*
- Waiving *in vivo* studies for monoclonal antibody biosimilar development: national and global challenges. *Dr Fiona Sewell, NC3Rs*
- Application of artificial intelligence and machine learning to improve 3Rs innovation. *Dr Avi Lerner, NC3Rs*

CRACK IT challenges

- Integration of the CRACK IT InPulse MuscleMotion® algorithm into the Clyde Biosciences Ltd CellOPTIQ® platform for functional cardiotoxicity assessment. *Dr Mark Bryant, Clyde Biosciences*
- Synthetic retina for drug discovery. *Dr Valeria Chichagova, Newcells Biotech*
- A human induced pluripotent stem cell (hiPSC) model for gene therapy vector safety evaluation. *Dr Michael Themis, Brunel University London*
- NeuroScreen-3D and NephroScreen: High-throughput toxicity screening and pre-clinical safety. *Dr Marianne Vormann, Mimetas*

External organisations

- Reduction and refinement of rodent juvenile toxicity studies – The simple approach to cross fostering. *Dr Malcolm Blackwell, Sequani*
- Optimal duration of non-clinical studies to assess the safety of monoclonal antibodies. *Dr Lolke de Haan, AstraZeneca and Dr Peter van Meer, Medicines Evaluation Board*
- Predicting the safety of medicines in pregnancy: A new era? *Dr Ross Hawkes, Medicines and Healthcare products Regulatory Agency*

NC3Rs-funded research

- Development of an adverse outcome pathway (AOP) for cardiotoxicity mediated by the blockade of L-type calcium channels. *Dr Luigi Margiotta-Casaluci, Brunel University London*
- Human *in silico* drug trials for evaluation of drug cardiac safety and efficacy. *Dr Elisa Passini, University of Oxford*

Annex 3: 3Rs challenge areas identified by delegates

Alternative systems and methods

- Can *in vitro* be even better/equivalent to *in vivo*? Should alternatives be benchmarked against *in vivo*? It may not always be possible/appropriate to compare new *in vitro* models to traditional *in vivo* models (e.g. personalised medicine).
- Organ-on-a-chip systems (or combinations of models). Are they fit for purpose yet? Would a test set of cross-pharma compounds help validate models?
- Could we develop a PK/PD model that could predict new exposures and assess if *in vivo* bridging work is actually needed (e.g. for repurposing, patent extensions, new route of administration, new indication etc).
- Where/when can alternatives be used? Can human-focussed computer models e.g. physiologically-based toxicokinetics-toxicodynamics (PBTK-TD) be used in place of *in vivo* bridging studies in toxicology?
- Are we ready for the potential replacement of animal experiments by human-focussed computer models: (re)training of scientists, impact on breeders, animal care technicians, consolidation of animal facilities etc?

Reviewing global practice

- Review of guidelines worldwide – differing regional requirements can increase the number/types of studies needed for global marketing. How can these be harmonized? e.g. China still expects acute toxicity studies for pharmaceuticals?
- Include Japanese Regulatory Authorities (PMDA) (and other regions) in discussions and working groups, to gain wider awareness and support for 3Rs initiatives to avoid additional studies for the PMDA.
- Can we stop doing 9-month non-rodent studies and do 6-month studies only instead?
- Revision of ICHS7 safety pharmacology guidelines – with recent publications questioning the value of respiratory and CNS studies, as well as the upcoming changes to cardiovascular requirements and wider inclusion of endpoints into toxicology studies, is it time to review and revise the guidelines?
- Bitterness testing is a niche test late in development, but the regulators say the best test is performed during human clinical trials. Can we avoid these animal studies?
- Rabbit pyrogen assessment – should be done only if required, after a monocyte activation test and a paper based risk assessment has been done.

Toxicology assessments and study designs

- Better design of GLP pivotal toxicology studies (e.g. 28 day). Use artificial intelligence (AI) to address dose groups (numbers, sizes) and doses.
- Relevant dose selection. e.g. immunogenicity driven exposure loss for biologics (e.g. mAbs) in NHP. Can we use non-GLP data to show we can expect to lose exposure such that there is no point including a "low" dose group?

Annex 3: 3Rs challenge areas identified by delegates

- Group size: can we reduce the number of animals on non-rodent chronic studies? Do we really need 4+4? Can we use 3+3?
- Do we always need maximum tolerated dose, dose range finding, 4 week, 13 week, 28 week etc.? Can we skip the sub-chronic toxicology studies (~13 week duration) and go directly from short toxicology studies (4-8 week duration) to chronic toxicology studies (26 week duration)?
- Species selection – how to standardise across the industry to ensure robust justification. Use of minipig as non-rodent – is it being considered during species decision-making?
- Multiple species tested in early PK studies for clinical dose assessments. Can we minimise the number of species tested?
- Explore the need for spare animals. How often are they really needed? A large historical control data repository would be helpful to support smaller group sizes if there are unexpected loss of animals, and mitigate against "spurious" effects.
- Re-use of animals, particularly NHPs previously exposed to biologic drugs- are there any circumstances when this would be OK? E.g. for non-terminal studies, can NHPs be re-used (PK or telemetry colonies plus others?)
- Explore the value of urinalysis – particularly with respect to rodents (especially mice) – as these collections are invasive or involve single housing. Should it only be included for specific studies when an effect is anticipated, rather than every study?
- Fasting or non-fasting for clinical pathology assessments?
- Are stand-alone safety pharmacology studies necessary or can we include these (and genetic toxicology) parameters into general toxicology studies. Can we remove respiratory studies from core battery?
- Could single species be enough for reprotoxicology studies?
- Usefulness of NHP ePPND studies – does data translate to allowing women of child bearing age to use a drug?
- Group housing: Can we house rodents in groups for embryo-foetal development/prenatal studies? E.g. where significant effect on food intake is not expected or group food consumptions would suffice?
- How can we encourage better uptake of microsampling and improve analytical techniques to enable the reduction in blood sample size for different purposes, e.g. haematology and clinical chemistry?
- Can the NC3Rs help capture trends in animal use in packages for "new" modalities? Focus on human relevance and selection of animal models and tailored data packages?
- Microsampling appears to be an appropriate refinement for juvenile toxicity studies, smaller samples could minimise the number of animals required for toxicokinetic satellite groups. However there seems to be limited demand and/or analytical methods available for this method across toxicology studies in general, the reasons for which are unclear.