WORKSHOP REPORT

Improving Science Quality through the Replacement, Reduction and Refinement of Animals in Biomedical Research and Development

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Improving Science Quality through the Replacement, Reduction and Refinement of Animals in Biomedical Research and Development

A workshop organised jointly by the Science Europe Medical Sciences Committee and the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)

Introduction

The use of animals in biomedical research and development, aimed at understanding disease and creating new treatments, is increasingly coming under scrutiny from the general public. A recent European Citizen's Initiative entitled 'Stop Vivisection', seeking to ban the use of animals in such research, was signed by 1.2 million people.[1]

In 2010, the EU adopted Directive 2010/63/EU to strengthen and harmonise legislation regarding the use of animals in research and to improve their welfare. This Directive was anchored in the principles of the '3Rs': replacement, reduction and refinement. Replacement refers to alternative methods that avoid the use of animals absolutely. Reduction occurs when researchers are able to obtain comparable levels of information from fewer animals, often through improved experimental design and techniques or statistical analysis. Refinement refers to improvements in scientific procedures and husbandry that minimise pain or distress and improve welfare.

Science Europe recognises both the ethical principles for reducing animal experimentation and the need for researchers to engage in the debate and to accelerate the implementation of the 3Rs in Europe. In this context, the Science Europe Medical Sciences Committee and the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) co-organised a workshop on 21 and 22 September in Brussels, entitled 'Improving Science Quality through the Replacement, Refinement and Reduction of Animals in Biomedical Research and Development'.

The aim of the workshop was to explore some of the key issues relating to research involving animals in the context of the 3Rs, with the overall goal of improving the quality of science in biomedical research and development, and to progress the 3Rs. Topics included barriers and misconceptions that impede implementation of the 3Rs, tools to support the better design of experiments, and examples of how new technological and scientific approaches may contribute to the 3Rs. The workshop consisted of a number of presentations by a range of experts in various aspects of research and of the 3Rs, followed by a general discussion.

The workshop did not aim to arrive at specific recommendations or conclusions, but was intended to raise awareness, stimulate debate and highlight key issues.

Linking Good Science and Animal Welfare

How Animal Husbandry Affects Animal Welfare and the Validity of Animal Research

Professor Hanno Würbel (University of Bern, Switzerland), spoke about animal husbandry in laboratories. There has long been debate about whether or not the way animals are housed impacts upon research outcomes.

A key consideration in animal housing in science has always been standardisation: ensuring that the environment and conditions in which animals are housed in one laboratory can be reproduced in another. This has resulted in spartan cages, largely devoid of what may be considered 'extraneous' material. Laboratory mice may show heightened levels of abnormal behaviour (such as hyperactivity) in these environments, but the success of breeding among them suggests that they adapt to these environments and that their conditions do not impact on the reliability of scientific results obtained with them.

Nevertheless, it is an ethically-desirable goal to have happy animals and to improve their welfare through good husbandry. However, there is a question over whether providing laboratory mice with materials for nesting, burrowing and so forth compromises the reproducibility of experiments. A comprehensive, multi-laboratory study that investigated the effects of this type of enrichment of the environment of laboratory mice showed that it affected neither precision nor reproducibility.[2]

In fact, in cases where different laboratories rigidly attempted to reproduce standardised housing conditions, significant variations in test results were observed. This study therefore suggests that standardisation, or attempts at achieving it, may itself cause variation and thus be a fallacy; heterogeneity in animal housing improves reproducibility, and enriched environments that produce happy animals are not detrimental to science.

Assessing and Alleviating Pain in Experimental Conditions – a Paradigm Shift

Professor Paul Flecknell (Newcastle University, United Kingdom) presented the subject of pain alleviation in animals in research and how to assess their level of distress.

Until the 1970s, it was considered both impossible and undesirable to administer pain-killing drugs to laboratory animals after surgery, as prevailing views included the notion that pain experienced by animals was not as severe as that experienced by humans and that the administration of analgesics could render test results useless, as the drug could interfere with multiple physiological pathways within the animal. While the latter might still be true in certain circumstances, this largely depends on the choice of analgesic, of which there are many.

There has historically been little work on what compound to give to animals, and in what dose, resulting in large variations between different laboratories. In recent years, researchers have begun to refine existing pain-assessment methods and to develop new ones; this is fundamental to our understanding of how to reduce pain and improve welfare.

These new pain-assessment methods include quality-of-life measurements; the amount of pain an animal is in is reflected in its behaviour and its day-to-day activities, such as nest building or burrowing. It is also becoming apparent that pain can be reflected in an animal's facial expression: 'grimacing'

can be observed in rabbits and rodents, and so-called 'pain face' or 'grimace' scores have been developed to measure the effectiveness of analgesia following an operation.

It is not possible to derive a standard dose for analgesia for a given animal in a particular circumstance; for some animals drugs work well, while for others they appear to be ineffective. Researchers therefore need to use real-time pain assessment to arrive at the appropriate dose for a given analgesic. While the number of researchers active in this area is increasing, there is still a lack of suitably-trained personnel for this newly-developed method. There are also resource issues that need to be addressed, as it is time-consuming, and thus expensive, to assess pain in a single animal.

More work is required in this area to improve the understanding of pain relief in laboratory animals, and, importantly, there is a need for improved education, so that people know what information is currently available and how it can best be applied.

Improving the Design of *In Vivo* Studies

Impact of Poor Experimental Design

Professor Malcolm Macleod (University of Edinburgh, United Kingdom) spoke of the impact that poor experimental design has upon studies involving the use of animals.

He pointed out that it is not ethical to carry out research that uses animals if the methodology of the research is flawed in such a way that no meaningful results can be obtained from the research. Systematic analysis of papers reporting on *in vivo* studies has shown that much of this work is at a high risk of bias, as a result of being underpowered, poorly randomised or insufficiently blinded, with many studies not reporting on these attributes at all. It also shows a negative correlation between the impact factor of the journal in which the study is published and the level of randomisation.

There is a strong case to be made for generating mechanisms in the peer-review process to ensure that methodologies are appropriate, and for institutions to promote rigour in this area. Like all research, studies that involve animals should have rigid protocols, defined before the study commences, and they should state exactly how many animals will be used, in order to avoid the risk of more animals being included in the study if the 'desirable' result is not initially achieved.

Tools to Support Better Experimental Design and Reporting

Dr Nathalie Percie du Sert (NC3Rs, United Kingdom) added more examples of insufficient reporting on *in vivo* studies.

She referred to a 2009 review by NC3Rs researchers on the quality of experimental design and reporting in 271 published studies that used rodents or non-human primates.[3] None of the papers reviewed described how the number of animals (the sample size) was calculated, and only 70% of the studies described a statistical analysis and presented the results with a measure of variability. In a quarter of the studies, there was no description of the animals' characteristics, such as their weight and age, and only 59% of the papers stated the hypothesis underlying the experiment and the number and size of animals used.

In order to improve the reporting of research using animals, the NC3Rs has developed the so-called ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines.[4] These guidelines are intended to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies. The guidelines were published in the online journal PLOS Biology in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies.

Copies of the guidelines have been sent to 30 countries and the website has been visited by people from 110 countries. The NC3Rs has been working with journals to incorporate the guidelines into instructions for authors. To date, over 500 journals have signed up to the ARRIVE guidelines.

The NC3Rs is also developing an interactive web-based tool called the 'Experimental Design Assistant' (EDA) which guides researchers through the design of their experiment. The system provides feedback on the proposed design and includes dedicated support for sample size calculations, randomisation and blinding. The aim is that by running their design protocol through the EDA, researchers can be confident that they have considered their experimental design in detail and that their methodology is sound.

Applying the 3Rs to Disease Models and Safety Studies

The 3Rs in Thrombosis Research

Dr Michael Emerson (Imperial College London, United Kingdom) informed participants of his experience with animal testing in cardiovascular research, specifically the process of thrombosis, which is a key event in heart attacks and stroke.

Thrombosis, or the formation of clots in the blood, is driven by platelets. In order to understand the underlying mechanisms of this process and to develop effective anti-thrombotic agents, we need to know more about how these platelets function. While *in vitro* assays exist to investigate the activation of platelets, the system is very complex and involves many other factors in the blood, which currently makes it necessary to work with animal models. The standard *in vivo* test involves the injection of clotting agents into the tail of a conscious mouse, with the end-point of the assay being the mouse's death.

Scientists at Imperial College London, with funding from the NC3Rs, have developed an alternative test system; radioactively labelled platelets are introduced into the mouse's blood under general anaesthesia, and low, non-lethal doses of clotting agent are then injected into the anaesthetised mouse. The accumulation of the labelled platelets can be monitored, and in this way researchers can measure platelet aggregation in response to the dose of clotting agent. This new approach reduces

both the suffering of the animals and the number of animals required; because a fatal response is not being induced, repeated experiments can be done on the same animal.

The team has shown that by using this new method, information that previously required 200 mice can now be obtained with 30. Not only is this a significant reduction in number of animals needed, but the method also provides better results and richer information, while at the same time reducing stress and pain inflicted on the animals, as these refined procedures are conducted entirely on mice under general anaesthetic – something that was not possible with the conventional method.

Organ-on-a-chip Technology

Dr Anja van de Stolpe (Philips Research Institute, the Netherlands) said that although human cells have been cultured in test tubes for many decades, technology is now advancing towards the creation of *in vitro* systems that contain multiple cell types interacting in complex systems, which are at least partially akin to human organs. For example, a consortium, led by the Philips Research Institute in the Netherlands, is making progress on growing human blood vessels in a laboratory, using human endothelial stem cells, which can be induced to self-assemble into a distinctive vessel.

Dr Van de Stolpe showcased the organ-on-a-chip technology that is being worked on at the Dutch hDMT institute.[5] They are culturing heart cells on a chip, with the system being engineered in such a way that the cells can be cyclically loaded, in much the same way that functioning heart cells are periodically loaded and relaxed in a beating heart. The team have also had encouraging results with the growth of networks of nerve cells on a chip.

Dr Van de Stolpe also showed an example of a similar development: at the Wyss Institute in Boston, United States, researchers have grown epithelial cells from human airways into layers on a chip, to create a potential assay system for common airway irritants, such as air pollutants and allergens. These models are being further expanded with other types of tissue, like muscle, blood vessels and fibroblasts, to simulate the human airway even more closely. Further refinements are being investigated, such as the possibility to load and unload these cells, (similar to the previously mentioned heart example) to simulate the stresses placed on them during breathing.

While animal models can provide important biomedical information on questions such as the efficacy or toxicological profile of a potential new drug, it is knowledge about the interaction of these substances with the human body that is ultimately required. As such, this type of technology may in the future help in further reducing the need for animal testing in biomedical research.

New 3-D Models for Drug Development

Dr Adrian B. Roth (Roche, Switzerland) offered participants a 'peek behind the curtains' at Roche, where researchers are working to develop three-dimensional cultures of human cells that can be maintained over extended periods of time for the development and safety assessment of new drug candidates. If such *in vitro* systems can detect toxicity earlier during the drug development process, this would result in the use of fewer animals.

The prediction of toxicity is notoriously difficult and is often not confined to single, simple mechanisms. Liver toxicity, for example, is typically complex and develops over time, involving the interplay of several cell types; it is often specific to a given species of animal as well.

The Roche team has developed a 3-D liver model containing a variety of cell types. Biochemical profiles of the model are promisingly similar to that of the human liver; the model has been exposed to drugs that have failed in the past due to liver toxicity, and it reacted in a way that is consistent with

the reaction of the human liver. Future research directions include the use of 3-D printers to engineer the models, which would remove the need for a scaffold for the cells to grow on; scaffolding can have an effect on the way that a drug interacts with the structure as a whole. It might eventually be possible to print organ models on a chip, or even to print several interconnected 'organs'.

Virtual Infections – *In Silico* Modelling of Leishmania

Professor Jon Timmis (University of York and SimOmics, United Kingdom) showcased a mathematical model of the infection and host response of the disease leishmaniasis, which is being developed by a team of engineers, modellers and immunologists at the University of York in co-operation with three SMEs in the United Kingdom, with an award from the NC3Rs' CRACK-IT programme for pre-competitive, proof-of-principle research. The disease, mediated by a parasite, kills around 40,000 people each year, mostly in developing countries. Over the past ten years, it is estimated that research into leishmaniasis has used some 40,000 laboratory animals.

The model, which will be deployed as a cloud-based service for people to access, is populated with data from the research literature (using only studies with robust methodology) and includes information relating to the impact of different therapeutic interventions, the parasite loading, biomarkers, and so forth. Users run a simulated experiment on the model, selecting the particular host, the organ of interest, the strain of parasite, and the intervention strategy, detailing a dosing regime. A 12-week simulation of the experiment can be run in around two minutes and indicates whether the experiment would provide useful information and how many animals would be required. In this way, experiments that would yield data of little or no value can be avoided, saving on the needless use of animals.

One of the main obstacles to the uptake of mathematical models is lack of transparency, which leads to lack of trust in the models. As such, a key aspect of working on this model is to ensure that it is transparent. SimOmics, one of the SME members of the team, has borrowed techniques from safety-critical sectors such as the aerospace industry to evidence the robustness of the model; at each critical point of assumption within the model, information is provided about where the data has come from and what evidence underlies the assumption. By ensuring that the model is transparent, the research team hopes to overcome reticence; users are able to interrogate the model exhaustively to satisfy themselves that it is fit for purpose.

3Rs Resources

3Rs Resources in Europe

Professor Maurice Whelan (European Union Reference Laboratory for alternatives to animal testing, Italy) presented the view of the European Union (EU) on the implementation of the 3Rs.

Professor Whelan noted that the EU has been strongly committed to the 3Rs for decades, reflected in Directive 2010/63[6] and in many other pieces of legislation that have an impact on animal welfare. The European Commission (DG Environment) co-operates closely with National Contact Points from Member States to implement provisions of the directive, which includes the development of guidance within expert working groups on topics such as education and training, project evaluation and retrospective assessment, and the severity assessment of animal procedures.

In its response to the European Citizens' Initiative 'Stop Vivisection', the Commission outlined in its June 2015 Communication several concrete actions it is pursuing to accelerate the implementation and increase the impact of the 3Rs in the EU.[7] Central to these actions is the aim to increase knowledge sharing between the many sectors and entities with expertise and activities in the 3Rs, to make better use of resources, and to maximise synergies and collective efforts. In addition to Commission efforts, it is important for Member States to agree on common challenges, strategic aims and objectives, and to tap into their available resources to support and implement policy decisions.

The EU Reference Laboratory for alternatives to animal testing (EURL ECVAM)[8] of the European Commission's Joint Research Centre has a long track record in the development and validation of *in vitro* methods for safety and efficacy testing of chemical substances and biological agents (such as vaccines) used in different sectors. Its mandate includes: promoting the development and use of alternatives in research and regulatory testing; co-ordinating validation of methods at EU level; facilitating exchange of information on the development of alternative approaches; providing public databases and information systems on alternatives; and promoting dialogue between legislators, regulators, and stakeholders.

In addition, a European research initiative to lay the foundations for assessing chemical safety without using animals is nearing completion. Over the past five years, a €50 million EU project on 'Safety Assessment, Ultimately Replacing Animal Testing' (SEURAT-1)[9] has seen scientists from 70 institutions work closely together to provide advanced alternative test systems, computer models, and public databases on chemical toxicity. These have all been brought together to form a testing platform that is being put through its paces in a series of case studies.

Points Arising from the Workshop Discussion

As stated above, this Science Europe workshop did not aim to arrive at specific recommendations or conclusions. The following points, questions and ideas that arose from the discussion at the workshop are solely intended to raise awareness, stimulate debate and highlight key issues for the implementation of the 3Rs:

- The 3Rs landscape across Europe seems to be fragmented and disjointed.

- There could be some value in the creation of a dedicated European centre for the 3Rs, which could organise symposia, fund PhD students, distribute knowledge and engage with the public, similar to the European Molecular Biology Laboratory (EMBL).

  Such an idea, or related initiatives, could be an effective way to promote the development and diffusion of new scientifically validated alternative methods. However, there are questions over

how such a centre would complement the role of EURL ECVAM, and whether it would need to be a funding body. The success of the UK's NC3Rs, or the support programme for alternative methods of the German Federal Ministry of Education and Research, can be attributed to their involvement in a wide range of activities, including their ability to give grants.

On the other hand, there could be a risk that such a centre might pigeonhole the 3Rs in a way that would be detrimental to the concept. The 3Rs should not be seen as a discrete activity in its own right; rather, it cuts across all areas of biomedical research and, as such, moves should be made to embed the concept in all biomedical research endeavours. A bottom-up approach might be more effective in encouraging the professional and learned societies across Europe to adopt explicit 3Rs policies and strategies, and to consider it a core part of scientific meetings.

- There could be merit in establishing a European mechanism to monitor current research practices and to test the effectiveness of interventions designed to improve research practice – not just in relation to the 3Rs, but more generally across biomedical research design, conduct and reporting.

- Although in some policy documents the 3Rs are explicitly described, in many declarations of strategy, practice and policy, they are merely implied. They should always be mentioned explicitly where applicable.

- For the 3Rs to gain traction, it is imperative that ethics committees, funding bodies and institutions insist that researchers who propose to work with experimental animals provide details of how their research will reduce or replace animals that might otherwise have been used, or how the experiment will be refined to improve welfare or reduce distress in animals used. A checkbox noting that the issue has been considered is not enough; quantifiable metrics should be provided.

- Research proposals must have rigorous methodology, with appropriate powering, blinding and randomisation. Given the high proportion of *in vivo* studies in which these attributes are insufficiently reported on, it is possible that far more animals are being used than necessary due to flawed methodology. Scientific journals have an important policing role to play in this regard.

- Achieving the goals of the 3Rs will require leadership: people with expertise in the 3Rs who are prepared to put themselves forward with energy, imagination and originality.

References

1. http://www.stopvivisection.eu/
2. Wolfer DP, Litvin O, Morf S, Nitsch RM, Lipp HP, Würbel H. (2004). Laboratory animal welfare: cage enrichment and mouse behaviour, in *Nature* 432(7019): 821-2. (http://www.ncbi.nlm.nih.gov/pubmed/15602544)
3. Kilkenny C, Parsons N, Kadyszewski E, Festing MF, Cuthill IC, Fry D, et al. (2009). Survey of the quality of experimental design, statistical analysis and reporting of research using animals, in *PLoS One* 4(11): e7824. (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0007824)
4. https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%202013.pdf
5. http://www.hdmt.technology
6. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063
7. http://ec.europa.eu/transparency/regdoc/rep/3/2015/EN/3-2015-3773-EN-F1-1.PDF
8. https://eurl-ecvam.jrc.ec.europa.eu, previously known as the European Centre for the Validation of Alternative Methods, which was established in 1991
9. http://www.seurat-1.eu/

Annex

Programme

21 and 22 September 2015 // Metropole Hotel, Brussels

Monday 21 September

20:00–20:30 Lessons Learned from Applying the 3Rs to Improve Asthma Outcomes
Professor Stephen Holgate, University of Southampton, United Kingdom

Tuesday 22 September

Introduction

09:00–09:10 Welcome
Dr Bonnie Wolff-Boenisch and **Professor Richard Frackowiak**, Science Europe

09:10–09:30 The 3Rs Challenge
Professor Stephen Holgate on behalf of **Dr Vicky Robinson**, NC3Rs, United Kingdom

Linking Good Science and Animal Welfare

Chair: **Professor Stephen Holgate**

09:30–10:00 How Animal Husbandry Affects Animal Welfare and the Validity of Animal Research
Professor Hanno Würbel, University of Bern, Switzerland

10:00–10:30 Assessing and Alleviating Pain in Experimental Conditions – a Paradigm Shift
Professor Paul Flecknell, Newcastle University, United Kingdom

Improving the Design of *In Vivo* Studies

Chair: **Professor Richard Frackowiak**

11:00–11:30 Impact of Poor Experimental Design
Professor Malcolm Macleod, University of Edinburgh, United Kingdom

11:30–12:00 Tools to Support Better Experimental Design and Reporting
Dr Nathalie Percie du Sert, NC3Rs, United Kingdom

Applying the 3Rs to Disease Models and Safety Studies

Chair: **Dr Bonnie Wolff-Boenisch**

12:00–12:30 The 3Rs in Thrombosis Research
Dr Michael Emerson, Imperial College London, United Kingdom

13:30–14:00 Organ-on-a-chip Technology
Dr Anja van de Stolpe, Philips Research Institute, The Netherlands

14:00–14:30 Virtual Infections – *In Silico* Modelling of Leishmania
Professor Jon Timmis, University of York and SimOmics, United Kingdom

14:30–15:00 New 3-D Models for Drug Development
Dr Adrian B. Roth, Roche, Switzerland

3Rs Resources

Chair: **Professor Stephen Holgate**

15:45–16:15 3Rs Resources in Europe
Professor Maurice Whelan, EURL ECVAM, Italy

16:15–17:00 Discussion, Round Up and Closing
Science Europe, **NC3Rs** and **Professor Stephen Holgate**

17:00 End of workshop

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