Meeting Report

The 3Rs Competence Centre (3RCC) – Better Research with Less Animal Testing?

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This question was one of the themes of the 11th Meeting on Animal Research held by Swiss Animal Protection (SAP) in Olten, Switzerland on May 18, 2018. This year, too, focused on the principles of the 3Rs (replace, reduce, refine the use of animals in research), looking in particular at alternative methods and the new national 3Rs Competence Centre (3RCC) founded in March of this year. The meeting, attended by some 100 participants, consisted of two thematic blocks: first, the structure, possibilities and planned development of the new 3RCC and other 3Rs centres in Germany (the Berlin-Brandenburg research platform (BB3R) and the Charité 3Rs Centre in Berlin) and, secondly, some of the latest developments in alternative methods presented by expert and eloquent speakers. These topics included toxicology without animal testing (Tox21 program), human lung cultures, alternatives to fetal bovine serum, recombinant antibodies, *in vitro* systems for testing antifibrotic agents, and microphysiological systems in applied research (organ-on-a-chip systems). The meeting concluded with a lively and informative panel discussion involving all speakers and members of the audience.

The 3Rs principles have been embedded in legislation with Art. 22 of the Swiss animal protection law for the past 20 years: Should alternatives to animal experimentation be available, researchers have to use them – in the view of the legislators. Even though the 3Rs have already advanced at the international level, there has been too little research, development, and utilization in Switzerland. Despite the recognized economic and scientific potential, not enough has been done with respect to more cost-effective and rapid alternative methods. Thanks to the 3RCC, changes in research and education will mean a reduction of animal testing in the future.

Dr **Chantra Eskes**, Director of the 3RCC, outlined the structure and scope of the tasks faced by the new center, in which the authorities, academic institutes, animal protection, and industry are involved. In 2016, the federal government commissioned the joint body of higher education, swissuniversities, to develop the concept for the new center. The federal council postulate report[1] published in 2015, based on an interpellation from the year 2010[2], was the starting point of this development.

Under the aegis of the University of Bern, the 3RCC will be promoted as a non-commercial research institute of national importance[3] and will also receive support from the State Secretariat for Education, Research and Innovation (SERI), the academic establishments concerned (mostly as in-kind contributions), Interpharma, the federal Food Safety and Veterinary Office (FSVO), and Swiss Animal Protection (SAP). Taken all together, the 3RCC will have about CHF 3 million each year to fund its activities – three times as much as was available to the former 3Rs Research Foundation. This means that the 3RCC will have the financial means to operate purposefully in promoting 3Rs principles in the three key areas of education, research, and communication. One of the center's immediate tasks will be to initiate and support the development of a national and international 3Rs network in order to increase researchers' awareness of the 3Rs and reinforce a 3Rs mentality. The network structure, centrally coordinated by 3RCC, is designed so that it can develop and act locally with the 11 university hubs but also involve industry, politics, and animal welfare in a targeted and sustained manner.

Speaking for the FSVO, Dr **Kaspar Jörger**, Head of the Division of Animal Welfare, highlighted the authority's high expectations of the 3RCC, especially concerning the three key areas of education, communication, and 3Rs research. The authority considers 3Rs training and continued professional development of research workers to be key elements of effective and sustained improvements for research animals and a reduction in the number of animals used. Close links with the universities should ensure that the 3Rs are introduced at an early stage in all natural science and medical degrees, with a direct effect on research and project results. Coordinated by the 3RCC, it should be possible to establish a sustainable 3Rs culture in Swiss animal research facilities, research institutes, and laboratories. The 3RCC should also become the know-how center for handling research animals in compliance with animal protection principles and a platform for the exchange of scientific knowledge and experience in the realms of the 3Rs. The 3RCC communication concept should provide a professional contact point allowing exchanges between all stakeholders (students, researchers, the general public, media, politicians, authorities, and industry). Active communication, both internal and external, greater transparency about animal testing, and the implementation of the 3Rs in the research and public interest communities are extremely important, also with respect to negative or "non-publishable" results.

1 Postulate *Zukunft Stiftung Forschung 3R und Alternativmethoden für Tierversuche*, submitted by the National Council Science Education and Culture Committee (SECC), Spokesperson Maya Graf, File number 12.3660, 17.8.2012.

2 Interpellation Maya Graf, *Schweizerischer Nationalfonds und Forschung mit Tierversuchen bzw. Alternativmethoden*, File number 10.3575, 18.06.2010.

3 Pursuant to Article 15 of the federal law on the promotion of research and innovation (FIFG).

Participants at the meeting also gained insight into networking with 3Rs competence centers in other countries and the exchange of knowledge, experience, and methods as several speakers contributed their own experiences.

In her presentation, Prof. **Monika Schäfer-Korting** described the Berlin-Brandenburg research platform (BB3R) affiliated to the Free University Berlin, which has been active in research and postgraduate education since 2014. The platform combines the regional 3Rs competences and promotes systematic research in this area. The postgraduate training course is the first in the world that offers a structured 3Rs qualification for young scientists, PhD students, post-doctoral researchers, and junior professors. The range of BB3R research includes models of skin disease, immunology, human-on-a-chip models, nanotoxicology, and *in silico* active substance analysis and drug design. Besides replacement methods, BB3R also conducts research and development into reduction and refinement methods. For example, Prof. Schäfer-Korting's R&D team is investigating the uptake and actions of cytostatic agents in tumor models of non-melanoma skin cancers and cancers of the head and neck. Translation of results from animal testing to humans is particularly limited and the BB3R aims to improve things through an integrated testing strategy. Potential drugs will first be investigated for suitability and tolerability on 3D models (i.e., without animal testing). Only successful effective substances will have to undergo subsequent tolerability tests on animals. Such procedures considerably reduce the number of animals required for testing.

Taking human lung cultures as an example, Prof. **Stefan Hippenstiel** presented the research being carried out at the new "Charité 3R – Replace, Reduce and Refine" center at the Charité – Universitätsmedizin Berlin, which was founded in 2017. Different approaches to animal experiments should lead to better therapeutic options and encourage a mind shift towards alternative methods. This aim will be supported by the interdisciplinary Charité 3Rs, working in close cooperation with other research facilities in Berlin, the pharmaceutical industry, and the state legislature to promote high-tech alternatives to animal testing. The center aims to make an international contribution to change the mind set in biomedical research and bring animal research studies properly into line with the 3Rs. The goal is to understand and diagnose human disease with innovative, human models and improve translation from bench to bedside. The BB3R and the Charité 3R form *Berlin 3R*, together with external partners who are indispensable for achieving their objectives. This requires the coordination and cooperation of academic partners, research institutes, local SMEs (small and medium-sized enterprises) and the local big pharmaceutical companies. A new animal-free research institute (*The simulated human*) at Charité Berlin has recently been approved with a budget of more than €34 million.

With the Toxicology in the 21st Century (Tox21) program, it was made very clear to the meeting that toxicology is also possible without animal testing. Since drug development with animal testing was becoming increasingly more expensive and many drugs have shown toxicity or been ineffective in humans despite prior animal testing, the US Academy of Science developed a new scientific concept in 2007. Experts believed that it should be possible to replace all routine and regulatory toxicity testing on animals with procedures using human cells and tissues in the near future. Multi-organ chips – cell culture chips that allow the simulation of human organs in miniature – are making a considerable contribution to this field. They are now regularly being used in drug development and the risk assessment of substances for use in cosmetics.

After in-depth examination of the Tox21 program, one study established that the concept had greatly improved the quality of risk assessment for healthcare and environmental protection (NASEM, 2017). At the end of 2017, the major US authorities introduced a comprehensive strategic and operational plan for the development of safe new drugs and chemicals (Thomas et al., 2018). It can be hoped that Europe and other industrialized nations will adopt this program, so that scientific advances in animal-free methods become widely implemented.

Prof. **Gerhard Gstraunthaler** introduced his audience to alternatives to fetal bovine serum (FBS). These alternatives are necessary because, apart from the methods used to obtain the serum (which are a concern to animal protection and should be phased out)[4], FBS contains an undefined mixture of biologically active substances with numerous disadvantages (including contamination with bacteria, viruses and prions, seasonal and regional variations in quality, etc.). Extensive batch testing therefore regularly precedes the use of FBS. In addition, the serum market is dependent on factors associated with the beef industry and it is even questionable whether the worldwide demand for FBS in research and biotechnology can be met in the future. Scandals about adulterated FBS have also reinforced doubts about the purity and quality of the sera. Despite these detrimental facts and the existence of many innovative approaches to the development of serum-free media, addition of FBS remains the procedure of choice in cell culture. Platelet lysates are a promising alternative and may be the answer to phasing out the use of FBS. The latest developments are in human platelet lysate (hPL), which has already been established as a fully-fledged substitute for FBS in many different culture systems. Production of these lysates is relatively simple and less prone to error. The starting products are expired units of donated platelets from blood banks. The procedure would provide a cost-effective and successful approach to replacing serum completely without any animal suffering (van der Valk et al., 2018).

In his presentation, Prof. **Pierre Cosson** informed the meeting that an animal-free technique to produce recombinant antibodies has been in existence for more than 20 years. It could greatly reduce the number of animals needed for experimental purposes and at the same time make research work much easier. However, the method has still not been accepted in basic

[4] Fetal bovine serum is obtained from the fetuses of pregnant cows. It is estimated that 800,000 litres of FBS are required to cover the global needs, which corresponds to about two million fetuses.

biomedical research, mainly because it is time-consuming and expensive. Since 2014, his group at a university center in Geneva has produced recombinant antibodies and made it possible for basic research groups and laboratories to access this technology. Hundreds of different recombinant antibodies are already available and held in a database. In the long term, the center would like to offer researchers international access to antibodies produced *in vitro* and hopes that the difficulties initially experienced with introducing the new and very promising technology will soon be overcome.

Antifibrotic agents are important for wound healing and scar formation. In particular, they are important for the recovery of the skin following injury but they are also relevant to fibrosis of the liver and lungs. *In vitro* systems for testing antifibrotic substances can replace animal models, which are anyway often unsuitable for such research questions. Wound healing and scar formation in animals differ somewhat from the inflammatory and healing processes in humans. Moreover, animal testing is time-consuming and expensive. Cell systems can be used for the preliminary testing of antifibrotic agents *in vitro*. Thanks to "macromolecular crowding", a synthetic cell culture, which represents the entire course of scar formation in a culture dish and can be used to investigate the biochemical and enzymatic processes, was successfully developed over 15 years. The *Scar in a Jar* system further developed and presented by Prof. **Michael Raghunath**, has been tested successfully with antifibrotic substances, making animal testing superfluous for this application. The pharmaceutical industry has now adopted *Scar in a Jar* for testing antifibrotics. Since 2011, GlaxoSmithKline has been using it for *in vitro* testing of substances to treat pulmonary fibrosis.

Prof. **Alexander Mosig** has been engaged in research on sepsis (blood poisoning) for many years, looking at the typical pathological changes and the loss of the gastrointestinal barrier functions associated with acute sepsis in humans. Various anatomical and genetic similarities between mice and humans, as well as economic considerations and factors such as low housing costs, high reproduction rate, and short life cycle in comparison with other rodents, have led to the mouse model becoming the preferred animal model for research into inflammation and infection. But the well-known differences between mice and humans (e.g., diet, habitat, body size) make the mouse model unsuitable for sepsis research. A microphysiological model of the human intestine and liver has therefore been developed to represent the complex changes and interactions between the two organs *in vitro*. The model can be used to examine the precise pathophysiological basics of organ failure in sepsis and develop new therapeutic approaches. This organ model and the associated organ-on-a-chip technology form the basis for the sustained replacement of animal experiments in sepsis research at the Jena University Hospitals and also in transregional research projects.

The panel discussion that followed again demonstrated the huge scientific potential of alternative methods and the 3Rs. Thanks to innovative approaches to research, many successful, reliable, cost-effective, and rapid procedures exist and are being further developed to reduce animal testing and the number of animals required, or even to make such testing superfluous. It often seems that the crux of the matter lies not in a lack of research or the development of new methods but in the lack of networking or communication within and beyond the animal research and 3Rs communities. In the future, gaps in 3Rs implementation have to be identified and filled, and more 3Rs centers instituted. The huge economic and innovative potential has to be made clear to the authorities and politicians so that efforts to establish 3Rs research and development do not fail through lack of financing.

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