

Meeting Report

The 3Rs and Replacement Methods – Better Research, Less Animal Harm

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The 12th Animal Testing Conference of the Swiss Animal Welfare Association (Schweizer Tierschutz, STS) was held in Olten on June 20, 2019¹. The principles of the 3Rs (replacement, reduction, refinement), in particular replacement methods and their developments, both at home and abroad, were the focus of this year's conference, which was attended by some 100 participants. Two thematic blocks highlighted both national and European developments towards enhanced research with, and on, the 3Rs, and provided concrete examples of how to gradually replace, reduce or refine animal experiments.

In Europe, there are only few countries that have not yet established a 3Rs center. Several countries even have several institutions that support the promotion, development and use of alternative methods and the 3R principles (replacement, reduction, refinement), many network on a transnational or international level. And yet, the number of animal experiments has remained high or even increased.

In 2017, some 615,000 animals were used in Switzerland in animal experiments – only 15,000 fewer than in 2016. In Germany, compared to the previous year, the number increased by 50,000 to a total of 2.8 million animals. In Austria, too, the number has risen since 2013 by 15% to almost 240,000 animals in 2016. Worldwide, well over 100 million animals are used annually for animal experiments. The overall numbers are increasing, partly because basic research with genetically modified animals is booming and partly because animal experiments are still the “gold standard” for many researchers.

The expectations of the 3Rs competence centers are accordingly high. The centers are also responsible for demonstrating greater transparency, especially regarding the implementation of the 3R principles. For example, it is almost impossible to find out how many animals have been saved by the 3R measures implemented to date, or how many animals now suffer less owing to refinement in terms of husbandry, handling, and testing.

The first speaker, **Luisa Bastos**, Animals in Science Program Leader at Eurogroup for Animals in Brussels, provided information on the developments and progress of the 3Rs in the EU. She explained that, even though there are positive developments regarding the 3Rs throughout Europe, these efforts are not yet reflected in a decreasing number of animal experiments. This is despite a growing understanding that animal experiments can often not provide results that are relevant and applicable for human

health. For example, animal experiments have still not succeeded to help achieve the development of new therapies for significant diseases such as cancer or Alzheimer's disease. The same applies to predictions concerning the toxicity of substances to humans, even though animal experiments are often required by law and must be carried out specifically for this purpose. In particular, the European Directive on the protection of animals for scientific purposes (2010/63/EU) provides fundamentally good prerequisites for the protection of laboratory animals; however, because implementation in the various EU states is insufficiently harmonized, it seldom leads to tangible or evaluable improvements or to a reduction of animal experiments. Political commitment and scientifically innovative research have recently shown that animal-free research is possible in many investigative areas. However, concrete, transnational measures on just how such scientific developments and strategies can be effectively established in the medium- and long-term have been, to date, largely lacking.

For more than 20 years now, the 3R principles have been specified in Art. 22 of the Swiss Animal Welfare Act and thus, if there are alternatives to animal testing, researchers must use them accordingly – this is the legislator's intention. Although progress has already been made at national and international level with regard to the 3Rs, also in Switzerland, and it is evident that substitute methods with proven scientific potential are often more conclusive, less expensive and faster, these have remained poorly investigated, developed and used. It is not yet known how much can change in the area of research and education with the help of the new Swiss 3R Competence Centre (3RCC) or, in the future, to what extent this could lead to a reduction in animal testing and the number of animals involved. Although the 3RCC has only been operational for about a year, the 3RCC Director, **Chantra Eskes**, already presented various 3R activities as well as its research funding program. In response to a first open call, 54 research projects were submitted to the 3RCC (of which 50% were replacement, 30% reduction, and 20% refinement project proposals). After evaluation by an external expert committee, six of these were selected for project funding and are now financially supported with CHF 1,265,000. The projects represent all of the 3Rs. In addition, a 3Rs Award was instituted, and the winner will be announced at the first Swiss 3Rs Day (September 2, 2019). The 3RCC is actively collaborating with universities and institutions of higher learning to design a 3R bachelor level program and a

¹ <http://www.tierschutz.com/tierversuche/tagungen/index.html>,
handout cf. http://www.tierschutz.com/tierversuche/tagungen/pdf/tagung2019_referate.pdf



3R e-learning module to promote the implementation of alternatives to animal testing. In addition, the 3RCC has initiated a Biostatistics Task Force to promote best-practice experimental setups, biostatistical procedures and provide the corresponding support. The 3RCC regularly publishes newsletters and is very active on its website and on social media. It also promotes the 3R principles by organizing and participating in scientific conferences at national and international levels. The 3RCC further supports the authorities in drawing up guidelines on alternatives to animal testing and offers assistance with the international regulatory implementation of non-animal testing methods.

Winfried Neuhaus, President of EUSAAT, Vienna, presented an update of the 3R network in Europe. The aim of the 3R network is to bring together the European 3R centers and associated companies in order to exchange ideas about best practice solutions, to improve communication, to promote the exchange of information, and to help prepare the groundwork for joint initiatives. An initial coordination meeting took place at EUSAAT 2018, and the follow-up meeting was held in Berlin in March 2019. Many ideas and suggestions were put forward by the network participants, and during the course of the discussions it turned out that the diversity of the members could actually prove to be the real strength of the network. In addition to their different structure and core areas of competence, they cover many different topics and employ experts in the various 3R areas. Furthermore, topics showing an urgent need for action were identified and assigned to specific task forces. The progress of these task forces was presented at the FELASA Congress in June 2019. The 3R network is an independent, open, free community in which 14 different EU countries are represented. Any 3R center or 3R company is welcome.

Kathrin Herrmann from the Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University, Baltimore, spoke about how to achieve a paradigm shift in animal testing and presented some of the contents of the new book she has co-edited². She explained the most critical shortcomings in animal testing, including that animal models often fail to protect humans and fail to be useful in developing new therapies. The wasting of resources for such research and the damage caused by animal-based research to both non-human animals and humans was discussed. The talk also dealt with the opportunities of working in science without animal testing, and of purposefully avoiding the existing hurdles to this, such as vague provisions on the replacement of animals in basic and applied research; the lack of courses on animal-free, human-relevant approaches; the inadequate financing for developing animal-free models and its anchorage in science. It was also shown that there is a demand for social action at the political level, as is currently also the case in Switzerland³.

Shirin Kadler, Technical University of Berlin, reported on the planned research complex in Berlin, The Simulated Human (Si-M), which will not perform any animal experimentation. The project is a unique joint venture initiated by the Technical University of Berlin and the Charité – Universitätsmedizin Berlin, which has a total budget of €34 million and shall open in 2023. It is conceived as an interface between the engineering sciences and medicine and will be realized in a spacious building, which is in part open to the public. Scientists from both institutions will work together in the building to simulate the functions of human cells and tissues using new technologies such as 3D cultivation, multi-organ chips, and 3D bioprinting. The elaboration of joint research projects will be funded by third parties. The coupled simulation of several human tissues opens up novel research approaches with significant clinical relevance, particularly in the field of cancer therapies and anti-infective strategies. The public will have access to two visitor floors and will be encouraged to participate in studies, e.g., by donating blood, or in debates and discussions. The Si-M research building is envisaged to act as a driving force for the upcoming paradigm shift in the medical biosciences.

Jan van der Valk from the 3R Centre at the University of Utrecht took over the continuation of one of last year's topics⁴, covering the production and use of fetal calf serum (FCS) for *in vitro* methods. Specifically, he discussed the contradiction that is posed by the painful serum collection from fetuses for use in methods intended to replace animal experimentation. FCS is a widely established additive to cell culture medium. It is obtained by heart puncture from bovine fetuses that are removed from the body of cows found pregnant during slaughter. This procedure is most likely painful to the animals. An estimated 500,000 liters of FCS from 1 million fetuses are used worldwide annually in research. Since it is a natural product with many components, the exact composition of FCS is not known and varies from batch to batch. This can affect research and testing as well as the reproducibility of the results. In contrast to FCS, chemically-defined media contain only defined amounts of known components. A publicly available database provides up-to-date information on serum-free media that has been developed for cell and tissue cultures. Several other products of animal origin are commonly used in cell and tissue cultures, e.g., trypsin.

The lecture by **Joachim Wiest** of Cellasys GmbH, Munich focussed on a microphysiometric alternative to the Draize test on rabbits. The painful Draize test is still used to identify chemicals that cause eye irritation although there is an EU-wide ban on animal testing for cosmetic products, their ingredients or raw materials⁵. Although numbers have decreased since 2010, more than 2,000 rabbits in the EU undergo this test as alternative methods

² Animal Experimentation: Working towards a Paradigm Change, <https://brill.com/view/title/35072?lang=en>

³ Animal testing ban initiative, <https://tierversuchsverbot.ch> and corresponding statement from the Swiss Animal Welfare Association, STS, http://www.tierschutz.com/tierversuche/stellungn_initiative.html, as well as the parliamentary initiative to ban animal testing by National Assembly member Maya Graf, <https://www.parlament.ch/de/ratsbetrieb/suche-curia-vista/geschaefte?AffairId=20180491>, see also the relevant animal protection background information at <http://www.tierschutz.com/tierversuche/schwerbelastende/index.html>

⁴ Cf. lecture by Gerhard Gstraunthaler, http://www.altex.ch/resources/Proceedings_STS_2018.pdf

⁵ Cosmetic Guidelines, https://de.wikipedia.org/wiki/Richtlinie_76/768/EWG_über_kosmetische_Mittel, cf. also Swiss Animal Welfare Association Report, Cosmetics without Animal Suffering, <http://www.tierschutz.com/tierversuche/kosmetika/index.html>

have not yet been validated to classify all categories of eye irritation. The progress of the microphysiometric alternative method was discussed.

Rehoming of laboratory animals has gained importance in terms of a “culture of care” for animals used in research and gives them a second chance at life after experimental testing. A rehoming project was launched in 2018 by the University of Zurich (UZH), the Swiss Animal Protection (SAP) and its section, *The Friends of Rats Club*, with the aim of rehoming animals that had been used in experiments to private placements. Not all laboratory animals can be rehomed owing to experimental and legal reasons. However, more than 80 rats have already been placed in new homes and it is planned that the project will be continued and extended to other animal species. The experimental rats are kept in groups at the UZH and are socialized with humans. The adopted animals are characterized by their good compatibility and their trustful nature. They are very suitable as pets and have been successfully placed in good living conditions with private owners⁶.

The ensuing panel discussion showed once again the huge scientific potential of alternative methods and the 3Rs and that, with

innovative research approaches, good, successful, cost-effective and rapid methods can be developed that reduce animal experimentation, and potentially even make animal experimentation superfluous. Often the crux does not seem to lie in the lack of research or development of the methods but rather in the lack of networking or communication within and outside the research groups. The gaps in the implementation of the 3Rs must be identified and closed more quickly in the future, also with the establishment of further 3R centers and with research centers that investigate human-relevant mechanisms using human-based methods. The enormous economic and innovative potential of the 3Rs, especially replacement methods, must also be demonstrated increasingly to authorities and politicians so that the important establishment of 3Rs research and development does not fail due to a lack of funding.

Julika Fitzi-Rathgen, DVM MLaw

Swiss Animal Welfare Association, Head of Animals in Research, Genetic Engineering and Veterinary Consulting Unit, Basle, Switzerland

⁶ Swiss Animal Welfare Association and UZH rehoming project: <http://adopt-a-pet.ch/rehoming-projekt/>