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Alternative Methods – Where Now? (2016)

The Quality and Validity of Animal Experiments (2017)

The 3R Competence Centre (3RCC) – Better Research with Fewer Animal Experiments? (2018)

Springer Spektrum

Dear reader

From the point of view of Swiss Animal Protection SAP, the reinstatement of the specialist conferences on animal testing, 3Rs and alternative methods has been well worth the effort. During the 1990s and early in 2000, SAP held a number of conferences on the subject of "Animal testing". Following a temporary break, this thread was picked up once again at the end of 2014, with the 8th Conference on Animal Testing, under the banner *Promoting alternative methods – replacing animal experiments*. The 9th Conference on Animal Testing, entitled *Alternative Methods – Where Now?* was held in 2016 and dealt mainly with the developments and advances in alternative methods. The 10th Conference on Animal Testing in 2017, under the heading *Quality and Validity of Animal Experiments*, considered a variety of questions and topic areas involving animal testing, the 3Rs, alternative methods and the balance of interests. It also dealt with the unavoidable extent of and deficit in the planning, implementation, evaluation and publication of studies involving animal experiments. The 11th Conference on Animal Testing focused on the presentation of new developments in the area of alternative methods, but also on structures, opportunities and the steps being planned for the new 3R Competence Centre in Switzerland (3RCC), as well as other 3R centres in Germany. These include the Berlin-Brandenburg BB3R research platform and the Charité 3R centre in Berlin.

Animal protection considerations have long since ceased to be the only drivers of the desire and demand that fewer animal experiments should be carried out for the purpose of research. Animal protection groups (including SAP) subjected animal testing to the scrutiny of society via three popular initiatives 25 to 30 years ago. Today's discussion about animal-testing-free technologies and their development concentrates primarily upon researchers, business, politics and the authorities. This situation is also reflected in the backgrounds of our speakers. However, this type of discussion among experts also radiates outwards into the whole of society – animal experiments have once again become a subject of public concern. This is particularly important for Switzerland. Although some progress in regard to the 3Rs has been made on the international level, it seems as if those very alternative methods that are more cost-efficient and faster, as well as having a proven economic and scientific potential, have hitherto been subject to a lack of research, development and exploitation in this country.

Publication of this edition of *ALTEX Proceedings* containing the speakers' papers from the three most recent SAP Conferences on Animal Testing (2016, 2017 and 2018) has been made possible thanks to financial support from the ProCare Foundation.

I would like to take this opportunity to thank all of those who are committed to the area of the 3Rs and alternative methods, whether through research, animal protection, the 3R centres and the authorities or by providing funding; no progress at all would be made without your energetic support.

Julika Fitzi-Rathgen, DVM MLaw
Swiss Animal Protection SAP

Presentations of the 9th Conference on Animal Testing

Alternative Methods – Where Now?

Hotel Arte Conference Centre, Olten, Switzerland
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Introduction

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The principles of 3R – Replacement, Reduction and Refinement – have still not established themselves to the extent that was envisaged by legislators as far back as 1993 and enshrined in the Swiss animal welfare law to promote and implement alternative methods. Despite the proven commercial and scientific potential of alternative methods, they are barely exploited at all in Switzerland. In 2014, more than 600,000 animals were used for experiments in Switzerland, and almost half of these (299,403) were used in basic research within the university sector.

Every year, far in excess of 100 million CHF of Swiss tax revenue is spent on animal testing – while just 400,000 CHF is channelled into 3R research and the development of alternative methods via the 3R Research Foundation, which was set up almost 30 years ago. This means that 99.6% of the available funding is spent annually on research with and/or on animals, while just 0.4% is invested in experiments that do not involve animal testing, despite the fact that the quality and significance of the latter are frequently superior.

The report published in July by the Swiss Federal Council in response to the postulate on the future of the 3R Research Foundation and alternative methods to animal testing does not disclose how many animals could have been spared by the 3R measures used to date. However, it does point to optional routes by which more intensive research, development and implementation of 3R methods could take place in future – assuming that the necessary resources were allocated.

The 9th Conference on Animal Testing focusses on the current position of science, research, industry and the authorities in relation to the future challenges of 3R – and alternative methods in particular – and how we can ensure that their results are monitored.

Animal testing belongs to the past – this is the future

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The use of non-human organisms in research and testing is one of the ways that has characterised the history of modern life sciences. The information produced as a result is seen as a guarantee of the wellbeing of humanity. Over the past six decades, many efforts have been made to design programmes in basic research and safety science that match technical and scientific achievements but are also ethically cost neutral for the user. The ethical costs are not just relevant for the human user, but also for the millions of exploited animals. As a result of these efforts, the challenging scientific sector that focusses on "Alternatives to testing on animals" has evolved so much that it is now ushering in a new era in life sciences. This new era is marked by a consideration of validity (e.g. reproducibility), by the use of human biomaterials (3D cell cultures, organoids and induced pluripotent stem cells (iPSC)), by the use of "high-content" methods (e.g. Omics), by the combination of computer-based approaches, such as "read-across" and "virtual organs", and by miniaturisation technology (organ/human on a chip). The rapid increase in national funding for this sector (e.g. in the USA, UK, China and Brazil) shows how the political arena is waking up to the enormous commercial potential of this field. The successes achieved in the area of "Alternatives to animal testing" will therefore open up robust opportunities for research and testing that is relevant to human beings in the foreseeable future and will remove the need to use animals in the life sciences.

Basic research, with and without animal models

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My presentation will demonstrate how modern, internationally competitive basic research is carried out in the life sciences these days. Good basic research, just like applied research and clinical research, is based on clearly defined working assumptions and research aims, and only differs from the latter in that the primary goal is to gain new knowledge and a better understanding of the fundamental biological processes. Importantly, we also need to acknowledge that the overriding majority of the necessary animal testing carried out in basic research is either not burdensome at all, or that it only has a minor impact (i.e. severity levels 0 & 1). Animal testing is only used so that we can study complex processes (e.g. embryo development and organ formation) that would be impossible to analyse in the absence of animal models; this is because of a lack of complexity or other shortcomings in the experimental systems. It is relevant to today's conference to note that practically all the research groups working in basic biological and medical research now carry out a significant part of their research using cellular and other systems (e.g. using organoids, "organs on a CHIP", iPS and other stem cells, human biopsies, etc.), as well as via experiments using animals. Complex processes and interactions are also analysed with the help of computer simulations within the system biology framework in order to gain new insights. Bioinformatics is rapidly gaining significance throughout the life sciences, enabling experiments to be far better targeted. Since all these non-animal methods and models are already a firm element of the research strategy, researchers do not usually point to them specifically as alternative methods, but simply regard them as an integral part of their chosen research strategy. I hope that this short presentation will provide you with an interesting insight into the methodology of modern life sciences.

The placental barrier: The use of new technologies and discoveries for meaningful human models

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The placenta's function as a supply centre and filtering unit, keeping the foetus away from pathogens and harmful substances, helps to ensure the optimum, undisturbed development of the unborn child. One important area of research involves an investigation into the transportation and effects of pharmaceutical products, environmental toxins and nanoparticles on the placental barrier. The aim of this type of study is to prevent any possible risk to the foetus (reproduction toxicology), and to make it possible for new approaches to treatment during pregnancy (e.g. nanomedicine) to be developed.

The human placenta is unique, with an anatomical structure and function that are very different from those of mice and rats. Human placental models are therefore absolutely essential if we are to obtain meaningful results. Nevertheless, many studies are still undertaken on pregnant rodents, as the available human placental models are either technically complex (*ex vivo* placental perfusions) or very much simplified (2D cell cultures, static transfer systems). The major technical and scientific advances achieved in *in vitro* cell cultivation now make it possible to develop innovative new human placental models, providing a better picture of the dynamic environment and/or the complex tissue structure, and they could thus contribute substantially to a reduction in animal testing.

The purpose of this presentation is to give you an overview of potential approaches to an improvement in the development of new human *in vitro* models of the placenta (as an example). Specifically, it will introduce you to the establishment of a perfused transfer model and to a 3-dimensional microtissue in the placenta. A comparison between these new *in vitro* models and the *ex vivo* perfusion system and simple 2D cell cultures should demonstrate which approaches (3D cultivation, co-cultivation or dynamic flow) can be used to significantly improve validity. Initial examples of applications using toxic nanoparticles show that the placental microtissues demonstrate higher resistance than 2D cell cultures, because of their tissue-like structure.

Organs-on-a-chip: An alternative to animal testing?

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Slide 1: Title

Ladies and Gentlemen, I am delighted to have this opportunity to present the results of our research in the area of *organs-on-a-chip* to you. I would like to thank the organisers of this conference, Dr Fitzi-Rathgen and Ms Landis, for their invitation.

Please allow me to say a few words first about the ARTORG Centre at the University of Bern: ARTORG is an abbreviation for "ARTificial ORGans". This centre was established some years ago, with the aim of improving co-operation between engineers and doctors in order to find solutions to clinical problems. The Organs-on-Chip Technologies group works closely with the pulmonary and thoracic surgeons at the University Hospital, with the aim of developing *in vitro* models of the lung.

Slide 2: Declaration of interests and the content of the presentation

Before I speak in greater detail about the subject itself, I would, for transparency reasons, like to tell you that I am also the founder of a start-up company called AlveoliX. This business was set up to market the organs-on-a chip in July of last year.

I will begin my presentation by talking about the crisis in the pharma industry, before I then define what we mean by "organs-on-a-chip". After this, I will introduce two examples of organs-on-a-chip – a lung on a chip and a microvascular pulmonary vascular model on a chip. Finally, we will look at the hope that is generated by this type of model, as well as the limitations and the outlook for the future.

Slide 3: The crisis in the pharma industry

The pharma companies currently face a major crisis because of the cost explosion experienced in connection with the development of new medical products. These days, we would assume that more than two billion dollars would be needed to fund the development of a new drug. Nevertheless, the number of new drugs approved by the authorities responsible for drugs in the USA, the FDA (Food and Drug Administration, USA) remains unchanged at about 20 per annum.

Slide 4: The development of new drugs

In order to understand why these costs are so high, it is important to know that the development process for drugs is extremely long, at 10 to 15 years. This process takes place in a number of different stages, and the pre-clinical and clinical phases are the most important of these. In the pre-clinical phase, a number of molecules are tested simultaneously *in vitro* and *in vivo* on animals. At the end of this initial phase, only a small number of molecules are picked out and approved for the clinical phase, in which they are tested on humans for the first time. Unfortunately, the success rate in this phase is pretty low, at just about 11%. This means that an average of 9 out of 10 molecules fail in the clinical phase, and the investments already made in them are wasted. This low success rate is specifically related to the pre-clinical testing models (*in vitro* and *in vivo*). These fail to deliver precise enough results, and it is therefore impossible to anticipate whether the molecules will have detrimental or beneficial effects in the human organism. Recently, this was once again shown very clearly in France, where a test subject died in the initial clinical phase and other people also suffered serious damage to their health.

Slide 5: Pre-clinical testing models

The most frequently-used *in vitro* models in the pre-clinical phase are based on a very old technique – the Petri dishes invented by Dr Julius Petri in 1887. In these two-dimensional Petri dishes, cell cultures are applied onto a hard substrate and covered with a physiological solution. This very simple environment in no way corresponds to the environment waiting for the cells *in vivo*. *In vivo*, the cells live in a dynamic, three-dimensional environment that exerts a powerful effect upon the cell itself and upon its behaviour.

On the other hand, *in vivo* tests that are often carried out on mice or rats do reflect the complexity of the organism. However, the results are not necessarily transferrable to human beings, since the differences between the species are considerable; the tests are also often carried out on young animals, whereas human patients are mostly elderly. Furthermore, beyond the purely scientific questions, we also have to take account of far-reaching ethical considerations.

One middle way that I would like to introduce to you today is the "Organ-on-a-chip". This involves highly advanced *in vitro* testing models by which the cell environment can be represented significantly more accurately than in a Petri dish.

Slide 6: Organs-on-a chip

What are organs-on-a-chip? The term "chip" comes from the field of microelectronics. The technologies used to manufacture the organs-on-a-chip are based on microelectronics and are equivalent to the technology used to manufacture the chips in our smart phones and computers.

This technology can be used very easily to produce extremely precise microstructures, such as microchannels that are as small as cells. These microchannels do not generally contain

microelectronic components, but it is very easy to establish cell cultures inside them. In this way, we can, for example, simulate the flow of blood in a cell layer or other elements of the cell environment. Thanks to this technology, we can also very precisely define what type of cell culture should be established in which location.

Slide 7: Lung structure

In collaboration with the pulmonary and thoracic surgery departments at the University Hospital in Bern, we at the University of Bern have developed several *in vitro* models of the lung, with the aim of reproducing certain lung diseases, such as pulmonary fibrosis and cancer of the lung.

The tree-like structure of the lung opens out into the alveolar sacs (sacculi alveolares), which is where the gas exchange process takes place. Oxygen enters the blood while CO_2 comes out. The alveolar structure is extremely sensitive and is similar to a sponge. A closer look reveals that the alveolar blood/air barrier is extremely thin, at just 1 or 2 micrometres. Here, epithelial cells are in contact with the air, while endothelial cells are in contact with the blood. If you could spread out the surfaces of all the 300 million alveoli (the air sacs in the lungs), they would cover an area as big as a tennis court.

Slide 8: Dynamic environment: the respiration function

This environment is dynamic. These photos clearly show that the shape of the air sacs changes between breathing out and breathing in. The mechanical strain produced by inhaling causes the alveolar calls to stretch out with every intake of breath. In normal breathing, this stretching effect amounts to approx. 5-12%. However, the level of strain can increase up to 20% if the patients are on a ventilator; this, in turn, can lead to lung epithelium damage.

Slide 9: Alveolar blood-air barrier *in vitro*

Using our knowledge of these parameters, we have now developed a lung on a chip. On this extremely fine membrane we see epithelial cells, which are in contact with the air, while endothelial cells are in contact with the blood. The fine alveolar blood-air barrier is emulated by a flexible, porous polymer membrane with a thickness of just 3 micrometres. Human lung cells can be grown on this membrane in order to simulate the alveolar blood-air barrier. This photograph shows one of these membranes, with a diameter of 8 micrometres. The cells grown on either side of this membrane are able to communicate with each other.

Slide 10: *In vitro* respiration: inspired by nature

In our emulation of the cyclical movements involved in respiration, we have taken our inspiration from nature. *In vivo*, the most important muscle involved in our breathing process is the diaphragm. The diaphragm contracts when we breathe in, and the chest expands. When we breathe out, the diaphragm relaxes and the volume of the chest decreases. *In vitro*, a flexible membrane is expanded cyclically in the lower section of a small cavity by an external pump that creates a vacuum locally. The movements of this micro-diaphragm are reproduced in the alveolar blood-air barrier. The cell cultures grown there are expanded in three dimensions – just as they are within the lung.

Slide 11: Lung on a chip or, more accurately, alveolus on a chip

This image shows a lung on a chip, with three alveolar membranes. Three chambers are filled with coloured liquids to make them more visible. Each of the alveoli is fitted with a flexible, porous membrane, upon which the lung cells are cultivated. The micropores are produced using the technique described above. They measure between 3 and 8 micrometres in thickness. In this video, you can observe the cyclical movements of the alveolar membranes, thanks in particular to the way that light reflects upon the membranes.

Slide 12: The breathing lung on a chip

This image shows an alveolar blood-air barrier in a confocal image. The epithelial cells are on one side of the membrane while the endothelial cells are on the other. The thin membrane (shown in black) is positioned between the two layers of cells. The video shows the epithelial "breathing" lung cells, which are subjected to a cyclical mechanical load.

Slide 13: Permeability

Thanks to this lung on a chip, we can investigate the effects of the mechanical load on the alveolar blood-air barrier. In the tests, the barrier was subjected to two molecules of different sizes – one of which was very small, while the other was very large. The tests were carried out statically on the first occasion and dynamically on the second. We breath in a large number of particles every day, and it is therefore important to know whether these have any effect on our lungs or other organs when they pass through the alveolar blood-air barrier.

The mechanical load did not significantly affect the transportation of the large molecule through the barrier. This means that the layer of epithelial cells remained intact, with no tearing. However, significantly more small molecules diffused through the barrier under a mechanical load. These results are similar to the test results produced by volunteer test subjects in a resting state and while carrying out a physical activity. In test subjects carrying out a physical activity, a significantly greater number of small molecules were transported than for the people who were at rest.

At present, several research projects are being undertaken with the chip. In particular, one project funded by the 3R Research Foundation is investigating acute post-traumatic inflammation of the lung. The reproduction of a model for pulmonary fibrosis is being investigated as part of a second project, financed by the KTI. The mechanical load seems to play an important role in the progress of this disease. Clinically-observed fibrotic damage does actually mainly occur at the edge of the lung, where the mechanical load is at its greatest. The aim of our study is to provide proof of this relationship.

Slide 14: Microvessels in the lung

The second model I would like to introduce to you today is the model involving microvessels in the lung. This photograph of lung microvessels shows the structure of the vascularisation around the pulmonary alveoli. This is precisely the aspect we would like to reproduce *in vitro*. For the model, we therefore replicated a small chamber with a diameter of 2 mm and a microstructure that was created with the help of technology from the microelectronics sector. A barrier was created by small lateral columns measuring 200 µm. The surface tension thus created prevented the ingress of the viscous solution through this barrier. Other channels were filled with a physiological solution and used to supply the cells in this hydrogel with nutrients and oxygen.

Slide 15: Video: Self-constituting cells!

In this environment, the cells contained in the gel self-constituted within just a few hours. The video demonstrates the course of this process over 72 hours; by the end of this time, we had obtained microvessels comprising endothelial cells and pericytes. The second video demonstrates that the microvessels are perfusable. It had never previously been possible to illustrate this *in vitro*.

Slide 16: Formation of microvessels

Upon closer study, we can see from this confocal image that the endothelial cells form a continuous, compact layer. From the other photographs, we can see that the presence of pericytes – i.e. the cells that stabilise the microvessels – is indispensable in order for the vessels to remain impermeable.

Slide 17: Vasoconstriction (narrowing of the vessels)

It is even more important, however, for us now to be able to test the function of these microvessels by injecting a drug that will constrict the vessels – such as phenylephrine. We can clearly see that the microvessels become considerably narrower within just a few minutes. We can also establish that those channels made up solely of endothelial cells without any pericytes do not react to the drug. It is therefore possible to use this model to test the vasoconstricting properties of a drug, so it could therefore replace animal testing for this specific question.

Slide 18: Conclusions and outlook

In general, we can say that organs on a chip open up completely new pathways for the development of new *in vitro* models, facilitating the reproduction of *in vivo* environments that have never previously been possible. The opportunities are therefore extremely wide-ranging, especially in view of the enormous progress being made in the area of pluripotent stem cells, which will be cultivated on these media in the future.

How great is the potential, therefore, for the replacement of *in vivo* animal testing? In general, we can say that every new *in vitro* method is capable of reducing the number of animal experiments. Our aim is to drive these efforts further forward and to develop a "Human on a chip" so that we can obtain systemic answers.

Nevertheless, we do need to bear in mind the wonderful complexity of the human body, which we anticipate will be difficult to develop as an *in vitro* model – if it can ever be done at all. In my opinion, the future lies in the development of *in vitro* models that deliver an answer to specific problems and could therefore help to significantly reduce the number of animal experiments.

Slide 19: Thank you

In closing my presentation, I would like to thank my team, who have carried out such outstanding work. I would also like to thank the doctors in the departments of pulmonary and thoracic surgery at the University Hospital in Bern and the many sponsors who have previously supported and continue to support our research activities. I would like to thank you too, for your kind attention!

Re-orientation of 3R research in Switzerland from the point of view of the Swiss Federal Food Safety and Veterinary Office FSVO

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Animal testing refers to any action in which living animals are used with the aim of testing a scientific assumption, obtain information, acquire or test a material or determine the effect of a particular action on the animal. Animal testing also refers to the use of animals for experimental research into behaviour and investigations in regard to education and training.

At the beginning of the 1980s, 2 million animals were used for animal testing in Switzerland. By the year 2000, this figure had fallen to about 600,000 test animals, and it has settled at around this level ever since.

Despite this stagnation in the number of laboratory animals, some progress has been made over the past few years in regard to the 3R principles. During the period under observation, the number of laboratory animals used per experiment approval fell by a third, which points to the successful implementation of the requirements for an improvement in animal experiments. This development was also confirmed in 2014. While the number of laboratory animals that were used rose by 3% in 2014 compared with 2013, the number of newly-awarded approvals for animal experiments has fallen by about 13%.

In 2014, most of the animals used for testing were rodents (78.8%). The other species of animals that were used were birds, domestic animals, farm animals, rabbits, amphibians, primates and other mammals. It is noticeable that the main increase has been in the number of poultry used in animal testing; this can be traced back to a behavioural study involving egg-laying hens, in which an investigation was undertaken into the effect of the way in which they were kept and fed. In addition, twice as many fish were used in 2014 than in 2013 (39,876 compared with 18,435). The reasons included a test involving fish, which investigated the effect of climate and water quality on the health and development of the trout.

The stresses caused to the laboratory animals are divided into 4 levels of severity – ranging from 0 to 3. Where the animal testing is at severity level 0, e.g. in tests involving the animal's food or the way in which the animal is kept, the animals are not stressed. On the other hand, animal testing involving a severity level of 3 is extremely stressful. In 2014, 77.4% of the animals were exposed to a severity level of 0 or 1 and 20.6% to severity level 2. 2% of the animals were subjected to a severity level of 3.

In 2014, almost half of the laboratory animals were used in basic research at universities and hospitals. This type of use of animals had thus increased compared with 2013 (+1.8%). Swiss industry in particular had used fewer animals (-3%). The number of genetically-modified mice increased by an overall figure of 5.7%. No animals were used for testing in the areas of cosmetics or tobacco products.

Nobody wants to undertake stressful animal testing. Unfortunately, animal testing is currently often unavoidable, e.g. for the approval of medicines or the evaluation of chemical risks, in order to assess possible health risks for humans and animals, or in basic research for "proof of concept" in the entire organism. Nevertheless, researchers have a duty to carry out any animal testing that is absolutely necessary in a manner that is as non-traumatic as possible.

The respectful, proficient, responsible handling of animals used for animal testing is not only an ethical and legal duty – it is also a precondition for any meaningful research involving animal testing. Animal testing is extremely costly – not least in terms of the required financing. The obvious conclusion is therefore that such testing should only be undertaken if it is absolutely essential in order to gain the anticipated scientific insight.

As far as the FSVO is concerned, all of the three Rs involved in the 3R principles (Replace, Reduce and Refine) are of equal value. We must do everything we can to replace testing on animals, develop alternatives to animal testing and minimise the number of animals being used for this work.

Research into replacement methods for animal testing (e.g. recombinant antibodies rather than monoclonal antibodies produced in a mouse) as well as research into alternative methods (e.g. computer models or *in vitro* techniques using organ-like tissue cultures from human beings) both present a major challenge, demand serious professional skill and require substantial funding over a long period of time. In addition, further animal testing is often unavoidable, e.g. in the development and validation of the replacement and alternative methods. As a result, it takes years for the implementation of this work to be reflected by a reduction in the numbers of animals used.

In addition to the development of alternative methods, a reduction can also be achieved in animal numbers by the acquisition of more information of a comparable quality from fewer animals (e.g. modern imaging techniques). The reduction principle also calls upon researchers to plan their animal experiments carefully, using appropriate statistical tools. This allows the number of animals that will be required to obtain a meaningful result to be estimated correctly.

As long as animal testing is being carried out, however, it is crucially important from the animal protection perspective to make full use of every opportunity to ensure that the laboratory animals are subjected to as little stress as possible (Refinement).

A direct and immediate positive influence can be exerted on the wellbeing of each individual animal used in animal testing by implementing stress-reducing and technical improvements. These include such measures as effective treatments for pain in laboratory animals, a standardised, centralised animal breeding system, optimisation of the conditions under which the animals are kept before, during and after the testing is over and the training given to researchers and the care staff in regard to the day-to-day care and contact with the animals during the animal experiments.

In its report in fulfilment of Postulate 12.3660 "The future of the 3R Research Foundation and Alternative Methods for Animal Testing", the Swiss Federal Council proposed certain measures to strengthen the 3R skills in Switzerland, including an expansion of the education, training and ongoing training of researchers in the 3R sector.

The Federal Council also proposed that a national 3R Competence Centre should be established, as a key measure towards strengthening Switzerland's 3R competence.

The FSVO has instigated the measures necessary to implement these proposals. For example, important decision-makers at all the universities and in industry have already indicated their willingness to include the subject of 3R in the curriculum for students at the Bachelor level on all natural science and medicine courses, and to put the relevant measures in place.

Significant steps have also been put in place for the creation of a national 3R Competence Centre. For example, representatives of various universities and technical universities from every region of Switzerland have assured the FSVO of their support and their interest in a national 3R Competence Centre.

At present, a working group is defining the remit of the future national 3R Competence Centre and drafting a more detailed catalogue of tasks and organisational structure (network) for this national 3R Competence Centre.

We can fulfil the requirement for animal testing to be limited to the absolute minimum and for the animals to be subjected to as little stress as possible by consistently applying all 3R principles of Replace, Reduce and Refine. However, serious efforts must be made to achieve a further reduction in animal testing and the number of laboratory animals. The potential for a reduction in the stress caused to laboratory animals is still far from exhausted. We must initiate the relevant research in this area too.

The reorientation of 3R research in Switzerland can succeed, on condition that we create and finance a national 3R Competence Centre with the following functions:

- The promotion of a 3R culture in all areas of research and animal husbandry
- The development of measures
  - To measurably reduce (or at least stabilise) the number of animal tests and/or the number of animals used in animal testing
  - To reduce the stress caused to the animals used in animal testing to the minimum
- The development and direction of a national 3R network (leadership) and integration into the international 3R community

3R Research Foundation – where now for 3R research?

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I would like to say the following in advance of this written edition of my presentation:

My statements are not the official opinion or attitude of the Board of Trustees of the 3R Research Foundation. Where this is the case, I will mention it specifically. My presentation is a careful personal analysis, undertaken on the basis of my experience and activities as a founder member of the Foundation, to inform those taking part in the conference at first hand. The President of the 3R Research Foundation, who is engaged in the Session of the Council of States and sends you his apologies, is aware of the content.

The written document of the presentation is divided into a main section and an appendix. In the main section, I introduce the Foundation and deal with current questions relating to Swiss 3R research, the future of our Foundation and a proposed 3R Competence Centre. In the appendix, I present my personal analysis on the subject of "Animal testing, alternatives and 3R research".

To some extent, my statements are critical because, ultimately, the future of 3R activities in our country is dependent on the will of the authorities and of industry to become still more involved financially. As in other areas, we therefore find ourselves facing the power (or maybe the impotence) of the facts!

"3R Research Foundation – where now?"

This was the interpellation submitted by National Councillor Maya Graf in June 2010 and answered by the Swiss Federal Council in August 2010[1].

On 17th August 2012, Maya Graf, in her function as the then President of the National Council's Commission for Science, Education and Culture, redoubled her efforts with a Postulate; under the title, "The Future of the 3R Foundation and Alternative Methods for Animal Testing", the Federal Council was asked to demonstrate in a report how the research into alternative methods to animal testing can be promoted and their implementation in research increased. *In particular, the Federal Council was required to demonstrate how the 3R Research Foundation could carry out its tasks more efficiently and effectively in future, and what measures would be necessary to make this happen*[2].

Profile and track record of the 3R Research Foundation[3]

The 3R Research Foundation is (like the Swiss National Science Foundation (SNSF)), an organisation governed by private law, rather than a state organisation, even though it is also supported by state funding. It is a collaboration between the parliamentary group concerned with issues relating to animal testing, Interpharma and the Animal-free Research Foundation. As well as representatives from politics, the pharma industry and organised animal protection groups, the Board of Trustees of the Foundation also includes employees from the authorities (FSVO) and scientific involvement. The Expert Committee is made up of university professors and specialists who are scientifically active in the life sciences.

This broad-based heterogeneous management committee represents the entire spectrum of interested parties and has concentred from the very beginning on pragmatically-oriented rather than ideological aspects of 3R. Three decades ago, we lived through emotionally-charged trench wars between animal testers and "anti-vivisectionists". In those circumstances, where different ideologies and interests simply clashed against each other, it was mainly a case of keeping a cool head and making 3R research in Switzerland acceptable to academia and the scientific community.

In line with the aim of the foundation to promote research in the area of alternatives to animal testing through financing, the 3R Research Foundation has supported more than 140 projects with an overall budget of CHF 18 million since 1987. This funding was made available in equal halves by the Swiss Confederation and by Interpharma. At first glance, this might seem a handsome amount but is very little at second glance. In 2013, the Swiss National Science Foundation supported projects to the sum of CHF 118 million, which flowed into open-ended research involving animal testing. For over 25 years, this has stood in contrast with the just about CHF 0.4 million in state funding available per annum for the promotion of alternatives to animal testing. See also the parliamentary interpellation of National Councillor Isabelle Chevalley[4].

Dr Hans Wyss, the longstanding Director of the former FVO and current FSVO also analysed the situation relating to 3R some years ago and lamented that the 3R Research Foundation received "too much to die and too little to live".

Animal testing can be replaced, reduced or refined. This is the meaning of the 3Rs in the name of our Foundation. The Foundation is explicitly named "3R", not "1R" or "2R". The 3R concept covers those principles that must lead the way where animal testing is concerned; if there is an animal-free method available

[1] http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20103576
[2] http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20123660
[3] http://www.forschung3r.ch/
[4] http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20143683

to investigate any question, then that work must be undertaken without animal testing. If animal testing is necessary and unavoidable within the meaning of the law on animal protection, the number of animals involved must be kept as low as possible. The third command requires that animals must suffer as little stress as possible during the experiment. The 3R Research Foundation supports research projects whose aim holds a promise of improvement within the terms of a 3R principle in comparison with current animal testing practice.

If we break down the project supported by the 3R Research Foundation according to the three categories of Reduce, Refine and Replace, the total of 142 research projects sub-divides as follows:

105 projects were financed in the Reduce category. This corresponds to 74% of all the projects. In the Replace category, the equivalent number was 50%, while 26% fell into the Refine category. As you can easily see from these figures, some projects were often assigned to more than just one single category.

In addition to financing, it is also important to have good project support, starting at the time of project selection and planning, and continuing to sustain successfully developed new methods until they are at the application stage. The 3R Research Foundation therefore includes a top-level twelve-person team of experts for this purpose. They enjoy excellent links within the scientific community and work under the scientific direction of Prof Ernst Hunziker.

Life Sciences incorporates an extremely diverse area of sciences, from physics, via chemistry, biochemistry and biology through to medicine. 3R-relevant research is also similarly subject-specific. Because of this high level of complexity, the scientific methods used within the specialist disciplines also vary so much that projects can only be promoted on a subject-specific basis. For this reason, the projects are evaluated by experts from the relevant specialist areas. Every expert on our committee is therefore networked with other external specialists.

The closeness of the 3R Research Foundation to the researchers

Here, I would like to examine the following statement made in Section 5 of the Federal Council's report:

> *"Because, specifically, of the current purpose of the Foundation and its activity profile to date, the 3R Research Foundation is not very suitable (or even suitable at all) for the function intended for the proposed 3R Competence Centre. In particular, it lacks the closeness to the researchers that would be essential for the assumption of such a function".*

In the following section, I shall not investigate whether the Research Foundation is suited to managing a Competence Centre. On the other hand, I will examine the assertion that our Foundation lacks proximity to the researchers. According to the Annual Report for 2014, at least 11 of the 15 members of the Foundation's committee of experts listed there are active in research at university institutions. The other 4 come from the FSVO, the organised animal protection sector and the pharma industry. 9 experts are university professors. This committee of experts has, over the past 10 years, evaluated over 300 research applications, the majority of which came from institutions in Swiss universities, for their scientific quality, practicality and 3R relevance. Behind each of these research applications is a group of researchers that has developed ideas, concepts and research plans for ways by which we could replace, reduce and refine animal testing. Our committee of experts has discussed methodical and conceptual problems with many of these project applicants and advised them on how they might structure the project applications so that they are even more 3R-relevant. The projects supported by the Foundation were provided with scientific support while they were carried out. Our Scientific Director made site visits to the relevant research institutions and maintained a dialogue with the project managers. The researchers submitted interim reports on an annual basis to provide an account of the progress of their project. A number of projects have also been cancelled. Could we keep our finger any tighter on the pulse of 3R researchers than we have already been doing for years?

The future of 3R Research with a national 3R Competence Centre

As I set out a year ago, at a FSVO workshop (*and this is my personal opinion, which I also share with other scientists*), I beg to doubt whether it would be possible for a national 3R Competence Centre to promote 3R research in a better or more targeted way, as outlined in Section 4 of the Federal Council's report. This is because:

1. 3R research cannot be designed in advance. Specific ideas for such projects arise in the minds of specialised research groups in this extremely heterogeneous area of Life Sciences, often as a side effect of scientific research into new discoveries.
2. The creativity of scientists should not be constrained by "dirigisme" on the part of higher-level institutions. In academic life, the bottom-up approach is superior to the top-down approach.
3. A 3R Competence Centre can never cover the broad diversity and heterogeneity of scientific activities in the Life Sciences. Prioritisation is also difficult in 3R research, and it may also hinder crucial research projects – because nobody can really anticipate or plan potential methodical breakthroughs in research. This represents a further argument for the bottom-up approach in research.
4. Bottom-up research in the area of 3R has made its most efficient progress in conjunction with independent financing institutions that are not themselves involved in research projects; there is a serious risk that a 3R Competence Centre would give preference to its own project ideas and disadvantage other approaches. The Swiss National Science Foundation, the European Science Foundation and the US National Science Foundation are all examples of independent financing institutions.

In Switzerland, the Swiss National Science Foundation would be the ideal financing institution, but the SNSF only supports knowledge-oriented research projects, not those that are method-oriented. A discussion on the 3R principles held on 2 Nov. 2012 failed to produce any softening of this attitude on the part of the SNSF.

In addition, there is one very important difference between funding for research from the Swiss National Science Foundation and that provided by the 3R Research Foundation: SNSF usually requires at least 4-5 years' experience of research, several examples as lead author of original articles in international scientific journals with a high impact factor plus authorship of a review to have any serious chance that the funding will be awarded.

The 3R Research Foundation imposes no such requirements; an application made by anybody may be approved by us, as long as it involves a good idea and the CV demonstrates an ability to publish. Neither impact factor nor age play any part in our decision. This means that young PhD and Post-Doctoral students have a chance with us, as do researchers from industry, private enterprise and start-up companies.

As a result, the 3R Research Foundation currently remains the only independent port of call for young scientists needing financial support for method-oriented 3R research projects in particular.

The potential functions of a 3R Competence Centre

I agree with the Federal Council's report that sustainable implementation of research results would be an important function of a national 3R Competence Centre, and this has hitherto been insufficiently covered in Switzerland. A national 3R Competence Centre could, for example, make a vital contribution to helping more methods and approaches with 3R potential that have already been published in a scientific journal to achieve a breakthrough. Specifically, this might be:

- Through the further development and validation of methods.
- Through their practical implementation and breakthrough, whether that might be in university research, the development of new drugs or new chemicals, or in testing procedures to analyse the toxicity or environmental impact of such products.
- This also includes the operation of a platform and a network for the exchange and dissemination of 3R-specific information within the scientific community, universities, industry and the approval and supervisory authorities.

The 3R Competence Centre and the future of the 3R Research Foundation

On 8 October 2015, our Scientific Director, Prof Ernst Hunziker was given the opportunity to present the position of the 3R Research Foundation to the National Council's Science, Education and Culture Committee SECC:

Here are a few important points from this presentation:

- *In the view of the Foundation, the measures put forward in the Federal Council's report (Clause 4.2) are to be supported. The envisaged national 3R Competence Centre could make it possible to achieve the critical size and recognition to produce the breakthrough for the implementation of the 3R principles on a broader basis.*
- *In this context, the 3R Research Foundation presents itself as the competent institution for the support of research. However, it must be provided with the necessary resources – more than the current sum of CHF 750 000.*
- *The 3R Research Foundation could be used in two different ways within the framework of a newly-created national 3R Competence Centre:*
  a) Clearly, the 3R Research Foundation could continue to support research into alternative methods to animal testing, including research into 3R methods.
  b) It is also conceivable that the Foundation could be used as the legal body for other functions included within the framework of a 3R Competence Centre.

Following the announcement made by the FSVO in December 2015 that the annual payments to the 3R Research Foundation would be stopped in 2017, any further discussion and appraisal of the proposals made about the future of the 3R Research Foundation in Section 5 of the Federal Council's report became redundant.

In as much as the planned 3R Competence Centre was intended to take up its work in 2017, the federal contributions for the present 3R Research Foundation would, namely, cease, because the FSVO would need the funds for the 3R Competence Centre. However, the absence of a federal contribution means that the requirement for payment from Interpharma is also unfulfilled.

The 3R Research Foundation will therefore have no income in 2017. Its activities will have to be abandoned and/or the Foundation will have to be liquidated. The remaining assets of the foundation will probably only just stretch far enough to cover the running costs for the current research projects and the liquidation.

For this reason, we have cancelled the impending call for project applications for 2016 with immediate effect and have communicated this decision accordingly.

Whether the Foundation will be liquidated or transformed into a new legal body is not ultimately within the decision-making remit of the Foundation's Board of Trustees. The power of disposal and the responsibility for any action lies with the Confederation and the new, revised deeds of the Foundation would have to be made available by the Swiss Federal Department of Home Affairs FDHA, as the supervisory authority. The new regulations of the Foundation would also have to be approved by the FDHA.

The fate of the 3R Research Foundation in future can be illustrated as follows: after 27 years of living as a caterpillar, the Foundation will pupate. In the most favourable scenario, it will then hatch out into a new and colourful butterfly – but in the worst case, it will not survive the pupation stage. The new sponsors of any future 3R Competence Centre will therefore, at the very most, bear only the name of the old Foundation.

Summary of conclusions

The 3R Research Foundation supports the measures presented by the Federal Council in its report. It is important for further progress to be made in regard to the subject of 3R. The question of whether the 3R Research Foundation survives or not is a secondary consideration.

The national 3R Competence Centre as envisaged could succeed in reaching the critical size and recognition necessary to achieve the breakthrough of the 3R principles on a broader basis.

It seems to me that the planned Competence Centre is mainly suited to:

a) Coordinating the 3R activities of science, industry and the supervisory authorities,
b) Facilitating the transfer of 3R expertise and exchange of knowledge, and
c) Purposefully helping to achieve a breakthrough in broader practice for approaches and methods that have already demonstrated their 3R potential, i.e. accompanying them along their final step towards execution and implementation, where the work not only involves the purely scientific aspects, but also legal issues and internationally interconnected interests in a globalised economy.

It is less appropriate, it seems to me, for any potential 3R Competence Centre itself to take over the financial support for research in 3R projects across the entire breadth and subject-specific, sub-divided depth as currently administered by the grant-awarding 3R Research Foundation; for reasons of scientific transparency, impartiality and fairness, this calls for a separate *independent* organisational structure, in the form that is already provided by the Swiss National Science Foundation, or even the 3R Research Foundation. A *separation of powers* is an absolute necessity between institutions that are active in the business of research and development on the one hand, and institutions that appraise, select and finance research applications on the other.

At present, our Foundation can (for financial reasons) only support one in 10 project applications in 3R research. If the 3R Research Foundation is to be liquidated in 2017 and if it still proves impossible to motivate the Swiss National Science Foundation support method-oriented 3R research in future, researchers at scientific institutions in Switzerland will find it even more difficult to carry out their particular 3R research project. In the event that the Foundation is liquidated, it should at the very least be ensured that we save its "crown jewels". Otherwise the researchers in this field could lose their access to the group of experts that provide them with a skilled discussions partner and a scientific network built up carefully over many years.

Appendix to presentation by P. Bossard:

Animal testing, alternatives and 3R research – overview and analysis

Scientific animal experiments originated at the very beginning of the natural sciences (in the modern era). As far back as the early 19th century, animal models were very popular in our attempt to understand human beings better. At that time, they were regarded as an ideal, successful representative model for human beings because we knew very little about the biological (and especially the physiological and biochemical) processes, and even less about their species-specific differences and similarities.

Animals were used at that time as the target organisms for a specific physical, chemical or biological effect. Even though people did not understand the processes thus triggered in their full complexity or detail (the laboratory animal was regarded as a black box), they could check the effect of these interventions on target organisms within parameters that were perceptive in relation to the status of science at that particular time. For example, an answer could be found, even in the 19th century, for the question about how much arsenic had to be swallowed by a mouse weighing 100 g to cause its death. Since then, however, our knowledge has improved enormously. Even by the middle of the 20th century, therefore, the limits of the transferability of results from animal testing to human beings were recognised and discussed.

Animal models as representative models for humans in the present

Nevertheless, animal models have not yet outlived their purpose as representative models for humans. The term "animal model" can be illustrated by the cancer or Parkinson's or diabetes mouse. Even before the advent of genetic engineering, such genetically inherited defects were systematically selected and "perfected" in laboratory animals (mainly mice) by classical breeding methods over many generations. These days, this work can be carried out much faster and more efficiently by direct interventions in the genetic material. Even today, the "good", newly-developed "alternative" methods are tested in validation studies by direct comparison with established animal models – in the absence of any better reference values and despite the limits to the transferability of results from animal testing to human beings having been demonstrated in the meantime.

Subjective social values and sensitivities also come into the equation here; these can be addressed under the "need for safety". Even though we know that there is no absolute safety, we want to reduce the safety risk (e.g. in the development of new drugs) practically to zero. The nearer we want to get to a zero risk, the more the associated cost, which increases disproportionately. However, we would prefer to accept a few too many animal experiments (than one too few). This practice is also supported by legal requirements (e.g. public liability).

These days, scientists can take advantage of far more refined methods than classical animal testing for specific detailed investigations. There are numerous alternative testing methods, i.e. many tests are carried out in a test tube, and *in vitro* in general. These methods can be made to measure, rather than using a large number of animal experiments, e.g. cell cultures, multicellular cultures, tissue cultures, whole blood cultures from volunteer donors, synthetic human skin, work undertaken on organ and tissue samples from operating theatres or from an abattoir, drug discovery by computer, serology methods for testing vaccines, refined laboratory and analysis techniques, more advanced planning of trials, the targeted use of statistical methods and much more. These types of alternative methods can, for example, be used to avoid the many animal experiments that used to be normal at the initial stages of development of a drug, during the pre-screening stage. In the pharma industry in particular, it has been possible to develop many new *in vitro* test procedures, with the result that there has been a reduction of about 60% in the use of animals in this industry over the past 10 years.

The amount of animal testing has decreased across the whole of Europe over the past two decades. In Switzerland, the number of animals being tested has fallen by three quarters, to 500,000 animals since 1983. Over the past decade, however, this downward trend has been halted in part by activities in genetic engineering research – while the systematic selection of mice with diabetes with the help of classical animal breeding methods were not counted in the animal testing figures, they are now included in the event of direct interventions in the genetic material (i.e. in the "construction" of mice with diabetes by genetic engineering).

The 3R potential of new investigation methods

There is some 3R potential in the natural science disciplines. 3R research as such does not really exist and 3R research is therefore to be found wherever the work involves animal testing. This type of 3R potential exists within different specialist areas, problems, applications and implementations. They are heterogeneous and highly diversified. In the past few decades, a host of new analysis and testing methods have been developed and refined in extremely varied areas of chemistry, physics, biology and medicine, and these have the potential to generate ever better and more reliable results, where the aim is to investigate the effect of substances on the human organism, or to research basic mechanisms in metabolic pathways and in diseases.

The limits and opportunities of alternative methods

Replacing animal testing by a method that removes the need for laboratory animals is perhaps the best solution, but it is not always possible. The "Replace" requirement is concerned with finding a replacement for animal testing – which is no easy task, given that a single replacement method can rarely act as a direct replacement for animal testing. However, one method or more in combination may perhaps produce enough information to remove the need for animal testing, or at least make it possible to reduce the animal testing requirement.

Cell and tissue cultures have already served us well in many instances. For example, we can now recreate a form of synthetic skin by using cell layers, and use this "skin" to evaluate the effect of potentially damaging substances. This is of interest to the cosmetics industry, for example. But tests involving cells, tissues and isolated organs have their limits, because it is impossible to investigate any complex phenomena of the intact body. In other, more direct words: cells don't suffer from anxiety or diarrhoea, and nor can you measure their blood pressure. By the time a newly-developed drug is ready to be clinically tested directly on humans for the first time, for example, our western society does not morally or ethically hesitate to test the drug on a different complex organism – an animal substitute – in order to fulfil our personal and social need for safety (we call this the internal human ethic).

As well as the generally recognised toxicity tests used to remove the health risk to humans (regulatory toxicology), a great deal of animal testing is also undertaken in biomedical research and in the development of active substances. The heterogeneous Life Sciences sector offers a broadly-diversified potential for the tailor-made replacement of animal testing by alternatives, thus leading to an improvement in scientific quality.

Impediments to the implementation of alternative methods

Many of these new types of "alternative" methods of investigation are cheaper than animal testing. However, the current problem is that such alternatives to animal testing (particularly the testing of materials (e.g. for their toxicity, carcinogenicity, teratogenicity, mutagenicity, etc.), have to be accepted by the approval authorities worldwide, after comprehensive validation procedures. Politicians (supervisory and approval authorities) find this extremely difficult, however, and prefer the well-established animal testing model. A variety of national requirements therefore hampers the implementation of new alternative methods in the globalised economy. As long as there are important markets whose national institutions make animal testing mandatory by law because of commercial interests (I am thinking here of Japan and the USA, for example) the longstanding animal models are not going to be discarded. In addition, the use of such animal models has, once again, gained a new impetus from the latest achievements in genetic engineering, not least as a result of economic considerations.

Furthermore, any laboratory involved in areas such as product-related quality assurance will not be keen to exchange trusted practices for new methods – methods that will initially lead to uncertainty and an increase in investment in terms of work and finance, before they eventually pay their way in the medium or longer term.

Evaluation and redirection of 3R research in Switzerland – from the political point of view

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Politicians have been concerned with animal testing and alternatives to animal testing for over 25 years. The promotion and support for the development of methods relating to the 3R principles were incorporated into the law on animal welfare as far back as 1991. The 3R Research Foundation was set up in 1987 and financed equally by the Confederation and Interpharma. The number of animals used in animal testing fell at first, from 2 million in 1983 to 566,000 animals in the year 2000. Since then, the overall number of laboratory animals has unfortunately increased continuously on an annual basis.

Industry uses ever fewer laboratory animals, but Switzerland's universities use ever more in basic research, "thanks" to new genetic engineering animal models. The number of animals more than doubled between 2000 and 2013. This growth gives us grounds for concern, especially as it is financed by public money, and innovation produced by establishing replacement methods would be appropriate. On the parliamentary level, I have put forward several proposals over the past few years (including 10.3576 Ip R3 Research Foundation – Where Now? 10.3575 Ip Swiss National Science Foundation and research involving animal testing or alternative methods, 11.1085 Survey on public money for animal testing – more transparency).

In 2011/2012, the National Council's Commission for Science, Education and Culture took up the subject on the occasion of the 25th anniversary of the founding of the 3R Research Foundation. On 17 Aug. 2012, it submitted Postulate 12.3660 on "The Future of the 3R Foundation and Alternative Methods for Animal Testing" and asked the Federal Council to demonstrate in a report how the research into alternative methods to animal testing could be promoted and how their implementation in research could be increased. The Federal Council's report has been available since 1 July 2015. The Federal Council also sees the need for action. It wishes to investigate the creation of a national Competence Centre, as well as stronger education and training for students and researchers on the subject of alternative methods, and to make the 3R Research Foundation more independent.

The National Council's Commission for Science, Education and Culture discussed the Federal Council's report on 8 Oct. 2015, in association with Hearings. Unfortunately, both a Commission motion for a national 3R research programme and a postulate by the Commission on the establishment of a Competence Centre for 3R failed by a narrow margin. We are afraid that, once again, nothing more will happen, even though there is a serious need to act. It cannot be right that 99.6% of the public funds for grants for research goes into experiments using animals, while the alternative methods receive just CHF 400,000 every year, with no new policy incentives. This must be put right in the ERI Dispatch for 2017/20. Politics, supported by animal protection groups and open-minded, innovative scientific circles must remain committed to this issue.

The attitude of Interpharma to animal protection and the promotion of the 3R principles within the industry

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The position of the industry

The pharmaceutical research industry is expressly committed to a respect for animals. Over many years in the past, pharma businesses have put in a great effort and achieved sustainable success, with a continuing reduction in the number of animal experiments and the stress they involve.

The research industry understands animal protection concerns and, through its support for 3R, it strives only to use animal testing to the extent necessary for the acquisition of scientific knowledge. Accordingly, stressful animal testing must be reduced to the absolutely essential.

Nevertheless, patients are entitled to be prescribed safe medication, and to the possibility of the development of treatments for the countless serious impairments, such as Aids, Alzheimer's, cancer and mental illnesses, for which products are currently either insufficiently effective or still completely unavailable. This demand cannot be met without animal testing. The pharma research industry therefore resists the imposition of partial or complete prohibitions against animal testing.

The challenges of research

Research and development make an important contribution to the improvement in medical care. Thanks to countless innovations over the past few decades, the pharma industry has been able to provide better products and services for the diagnosis and treatment of diseases. Nevertheless, there are still many illnesses that are either impossible to heal, or for which the treatments are unsatisfactory.

On the long road towards producing new drugs and treatments, many questions about new candidates for effective agents are investigated with the help of computer-based simulation models, or even *in vitro*, i.e. with cell and tissue cultures or with isolated organ systems. In addition to the classic cell lines, a number of new systems have also been developed to provide a more complex picture of vital lung or liver sections, for example.

Nevertheless, experiments on and with animals are often unavoidable. Even the most up-to-date technologies are still unable to represent living organisms in their entirety, or to provide a good enough picture of the interplay involving organs and organ systems. Where certain questions in basic research are concerned, therefore, animal testing continues to be as indispensable as ever. The appropriate use of animals also makes a vital contribution to ensuring that new drugs are safe and effective. Studies on animals deliver important information so that we can reach some conclusions about the way the human body will react. However, animal testing cannot answer every question. Nobody can predict with absolute certainty how a new substance will behave in the human body. Nevertheless, animal testing does allow us to calculate the risks for human beings.

Animal protection in the industry

Interpharma's member companies are expressly committed to a respect for animals and are guided by the 3R animal protection principles: Reduction, Refinement and Replacement (of animal studies). Implementation of the 3R principles is now a component of these companies' binding global animal protection policies. The companies therefore search actively for new and improved methods and techniques to reduce the numbers of animals required, to limit the stress caused to laboratory animals to a minimum and to replace animal testing to an even greater extent.

The support provided for the 3R Research Foundation over many years is a sign of the clear recognition by the industry that animal testing must only be undertaken to the extent that it is necessary for the acquisition of scientific knowledge. Stressful animal testing must therefore be limited to the level that is unavoidable. The search for alternatives to animal testing and the clear commitment to achieving a balance of interests has led to a massive reduction in animal testing, by over 60% to a current level of about 600,000 animals over the past 30 years.

Animal Welfare Charter

Five years ago, Interpharma introduced the animal protection charter. With this charter, the pharmaceutical research industry underlines its desire to honour its ethical obligation towards animal testing. The companies report on their activities and successes in regards to animal protection matters annually in their Animal Welfare Report.

One good example of the way in which the member companies cooperate with each other is provided by the joint audits undertaken at the breeding firms. These audits work towards the goal of discovering any defects in the animal protection area at an early stage and realising improvements on a partnership basis. This exchange of information should, at the very least, serve to ensure the optimum implementation of the minimum legal requirements, and simplify efforts beyond that minimum level towards the implementation of 3R. Cross-company for-

mulation of the relevant checklists, listing over 200 questions, policy statements and a set of joint regulations, has taken some time. Regular joint audits have been undertaken at international breeders since 2014.

3R in the companies

For the pharmaceutical research companies, all the 3Rs are of equal significance for animal protection. In some member companies, internal national and international 3R prizes are awarded regularly. Researchers from the different departments can submit their activities and developments and over the past few years, interest in taking part in the 3R awards has grown steadily. For example, one member firm has registered an increase of 30% in the number of projects submitted.

In one example of the work involved, a process has been developed in one company that now allows bile to be obtained from a dog using a capsule that the dog swallows, with a special thread that can draw up fluid, rather than invasively, through an incision in the abdominal wall and catheterisation. This makes it possible to avoid the pain and extended recovery time caused by surgery (Refine). Another research group is working on the phototoxic property of pharmaceutical agents. A new testing system has made it possible for phototoxic substances to be identified in the animal, even early on in advance of the testing stage. Fortunately, the new testing system has also been taken up in the safety testing procedures used in Switzerland, Europe, the USA and Japan. This recognition can lead to a massive drop in the number of laboratory animals required (Reduce). In some cases, animal testing can even be replaced completely; one example is an impressive model of human skin for research into vaccination against the bacterium *Staphylococcus aureus*, which is responsible for skin and muscle diseases in humans and also life-threatening illnesses such as inflammation of the lung and sepsis. Tests involving animals can now be superseded by this new model of the human skin in order to explore the operating mechanisms of different formulations for vaccines (Replace).

Dialogue with the animal protection organisations

Interpharma is also working on a dialogue with people involved in animal protection. For a good four years by now Interpharma has been involved in a dialogue with Swiss Animal Protection (SAP), and for some time, this dialogue has also included Animal Free Research and Zurich Animal Protection. The industry is more open now, and we appreciate this dialogue, which helps to break down mutual misunderstandings.

The future of 3R – the necessity for international networking

Interpharma welcomes the proposed Federal Council measures to promote 3R. Education and training of the researchers is undoubtedly the key to successful implementation of the 3R principles. Even now, researchers who are involved in animal testing undertake a theoretical and practical training course that lasts several days. Education can certainly be strengthened further by incorporating the theme of 3R into the curricula of natural science and medicine courses, where this makes sense. We also explicitly support the creation of the "3R Specialist" function within the research institutions. In the industry, this step has already become established under the name of Animal Protection Officer or Animal Welfare Officer, with very good results.

A national Competence Centre is being planned as a further measure to reinforce 3R research. The aim of this is to purposefully promote this type of research and to implement the relevant results sustainably in collaboration with the industry and the universities. The Competence Centre could supply services in the area of 3R education, training and ongoing training to the enforcement authorities, industry and the universities. Since implementation of the 3R principles must be carried out by the researchers decentrally and on site, any 3R Competence Centre should provide a supportive function for the researchers, helping them to research and validate 3R methods.

It seems to us that international scientific connections are the key to achieving the synergies that can also be found with industry.

We might mention the British NC3R as an example of this. This is a national 3R centre whose "Crack-it" programme pushes and finances collaboration and networking between academic and industrial 3R research. It is also worth mentioning the Basel Declaration. Over 3,600 researches throughout the whole world place the 3R principles at the heart of their commitment to responsible research with animals. Through its world-wide grassroots network, this association disseminates a 3R mentality among researchers, even in those countries that still have some catching up to do. In the European IMI project – the largest public-private partnership in the life sciences worldwide – 3R aspects are included in the topic selection process. In the "eTox" project, for example, innovative new software tools were developed so that potential toxicities in candidates for new drugs could be better predicted.

3R at the University of Zurich

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The University of Zurich is committed to a respect for animals, to the three "R"s (Replacement, Refinement and Reduction) and to exemplary implementation of the legal and internal requirements. Animal testing must only be used when it is absolutely indispensable for the acquisition of scientific knowledge. As required by law, it must be restricted to the absolute minimum. For certain problems in basic research, however, animal testing continues to be unavoidable, particularly when the concern involves the effect on the whole animal, or interactions between the animal and the environment, or between different systems within the body. These might, for example, be questions about cognition, behaviour or the immune system, but they may also be part of the development of new diagnostic options in veterinary science.

The UZH has been subject to a Policy Paper on experimental veterinary research for many years. Almost ten years ago, it also appointed an Animal Welfare Officer, in conjunction with the ETH. By now, the UZH has two Animal Welfare Officers, who examine each application to undertake an animal testing project. This avoids redundancies in intended applications, encourages the latest testing procedures and investigates researchers' intentions. In addition, the Animal Welfare Officers promote an exchange of information and expertise between the researchers. This type of exchange is also possible within the framework of the Swiss Network of Animal Welfare Officers. The Animal Welfare Officers are authorised to carry out regular internal inspections of animal husbandry and animal testing locations and to issue directives.

The Institute of Laboratory Animal Science at the UZH offers high quality courses in Laboratory Animal Science that are also attended by researchers from other countries. In 2016, they ran more than 50 courses. Outstanding education and training is thus available to all UZH researchers, and the UZH can, if necessary, work with the Institute of Laboratory Animal Science to offer training courses for specific specialist competencies. The UZH is also supporting the 3R concept by setting up the LASC (Laboratory Animal Services Center) and the accompanying centralisation of the animal husbandry function. Most of all, we would like to mention the "Minimum Standards", which go beyond the legally imposed minimum requirements; the additional training for researchers with regard to animal husbandry, the enrichment and conditions under which animals are kept or bred and the prohibition on certain types of housing, which may be legally approved but do not meet the "Minimum Standards". The presentation puts forward examples of 3R activities at the UZH and ideas about how the UZH could strengthen 3R still further.

The UZH is hoping for financial support from the planned 3R Competence Centre for projects in all three of the "Rs" – this is therefore not a matter of Replace alone, but also of Refinement and Reduction. The Competence Centre is meant to act as a centralised point for the collection, administration and dissemination of information on 3R, possibly via the existing networks and organisations (Swiss Association for Laboratory Animal Science, Swiss Network for Education in Laboratory Animal Science, Swiss Animal Facilities Network, Swiss Network for Animal Welfare Officers, etc.). At the same time, links must also be established with the 3R centres already established in other countries, such as the NC3R in the UK.

In this sense, the planned 3R Competence Centre is required to take on a significant communicative and consultative function. At the same time, it must not simply act as a platform, but must also identify unanswered questions of its own in the area of 3R, and work with partners to develop strategies. The introduction of 3R Awards for all three areas and the establishment of a 3R professorship would also be regarded as desirable activities for the Competence Centre.

Research without animal testing – from vision to reality

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This presentation will provide a brief look back at the history of the Animalfree Research Foundation, which celebrated its fortieth anniversary in 2016. This review will highlight milestones in the development of the 3R concept and the advances in the animal protection sector in Switzerland, as well as in other countries.

The report prepared by the Federal Council for Postulate 12.3660 was undoubtedly one of the most important events of recent times. The key points here include the establishment of a 3R Competence Centre, a strengthening of the available education and training and the publication of even those results that seem negative. The presentation will address ways in which these goals could be implemented in practice and/or whether they have already been implemented successfully in other countries.

One focus of the presentation will be on the education of the researchers, particularly with regard to publication and research in the literature for results that are relevant to 3R, and the improved implementation of statutory requirements in order to advance the effective, prompt implementation of methods that do not use laboratory animals.

Animal protection and alternative methods

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In the election battles about initiatives involving laboratory animals that took place more than 20 years ago, the politicians, authorities and research and science sectors convinced the voters with the argument that stressful animal testing, including the use of laboratory animals, would be drastically reduced in the medium and long term, and that alternative methods avoiding the use of laboratory animals would take their place.

Animal lovers and protectors currently feel deeply betrayed in their past faith. Since 2000, the number of animals used in experiments has risen by just about 7%, to a current level of 606,000. Nevertheless, we cannot blame the politicians for the two decades that have been lost from the animal welfare perspective. In the law on animal protection, Parliament outlined guidelines and opportunities for ways in which the universities and the economy, and the Federal Council in particular, might move away from stressful animal testing. For example:

Article 17 Restriction of animal testing to the extent that is absolutely unavoidable
Article 19 Option of prohibiting inadmissible experimental goals
Article 19 Prohibition on stressful animal testing if this leads to a comparatively minor increase in knowledge
Article 22 Promotion of the development, recognition and application of methods that replace animal testing

In relation to the use of laboratory animals, there has been a move away from industry towards university research. The numbers of laboratory animals have increased by 28% in the state-supported basic research sector since the year 2000. We fear that this count will increase again in future – REACH and nanotechnology will crank up the use of animals yet further, as will the development and production of genetically modified animals. For example, 950,000 genetically-manipulated animals (mainly mice) were bred in Switzerland in 2013, and a further 300,000 imported from foreign breeding stations. More than half of the animals bred in this way were produced in excess of what was required, however, and were mostly killed and disposed of without being used in any way at all.

Over the past few years, several universities have expanded their animal husbandry facilities, building massive new systems. Since the tax payer finances a considerable proportion of the cost of university research projects using animals, together with the investment in the construction of facilities for laboratory animals and the animals' upkeep, there is naturally great public interest in all this. It is not surprising that animal lovers and animal

protectors are not the only people who have a critical view of this development. The various university mass breeding stations (whether newly-built or still at the planning stage, and some of which house up to ten thousand rodents) consume horrendous amounts of funding for upkeep alone. The full cost of just 4,000 places for mice is about one million CHF every year. This amount of money could be used by a university to install a professorship in alternative methods, including a research institute.

On the whole, SAP is disappointed by the Federal Council's report on 3R, published in the autumn of 2015 as demanded by the Commission for Science, Education and Culture in 2012, and this must be stated quite clearly. The analysis provided in the report is largely viewed through rose-tinted glasses, and is contrary to earlier replies by the Federal Council to various proposals in this area. For example, the most basic aspect is not clarified at all, i.e. the volume of animal testing spared by the 3R measures brought in thus far. This means that there has been (and still is) no examination of their results, even though millions of Swiss Francs of tax revenue has been spent in this way. And their feeble track record with regard to the development and implementation of alternative methods is simply hidden. In the social context, the fact that over half the population regards animal testing as a necessary evil because there is supposedly no alternative is simply ignored, but over 2/3 of the people evaluate animal testing as cruel to animals, and even agree to spending 83% more tax revenue on the development and implementation of alternative methods.

The report also fails to mention the fact that certain experiments on animals can barely, if at all, be transferred to human beings, that about half the animal testing studies are flawed and fraught with errors, and that many thousands of animals are therefore sacrificed in Switzerland without any gain in knowledge or any benefit. To us, it seems particularly problematic that a report on 3R is specifically silent on the major scientific and economic potential of Replace, i.e. on alternative methods to animal testing.

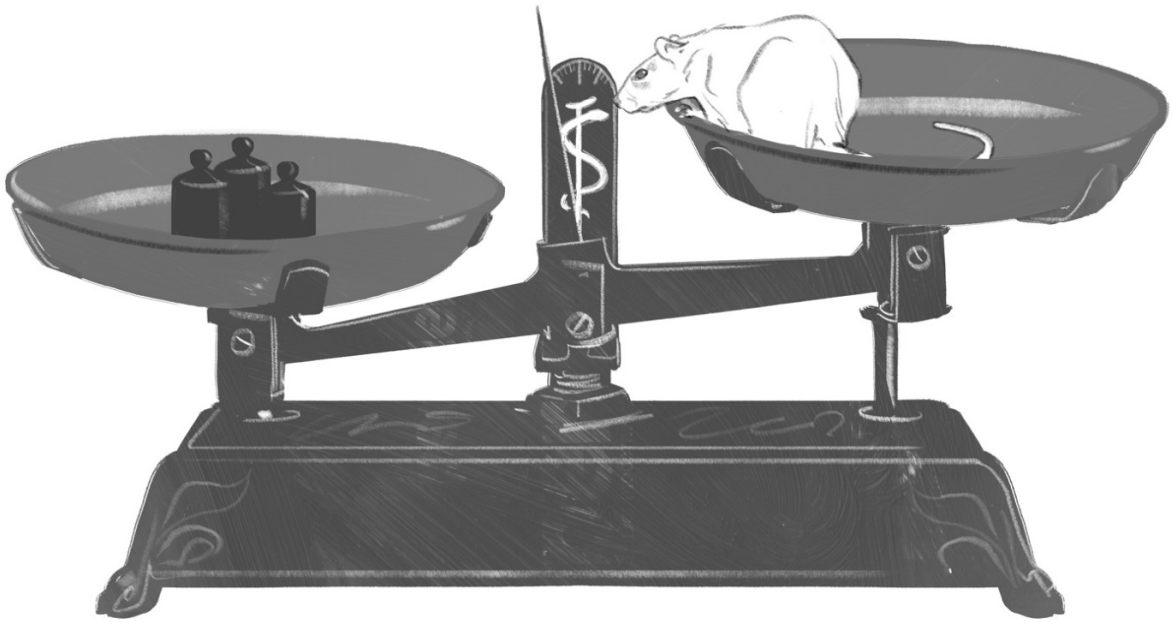
In our opinion, this all fits in with the inexplicable restraint of the Federal Council, particularly in connection with basic research involving animal testing undertaken in the university sector. In the spring of 2015, for example, an opportunity arose to initiate a national research programme on alternative methods. There was no lack of practical submissions from universities, researchers and the FSVO, or serious advocates from civic and red/green political circles – even the pharma industry were on board. Only the Federal Council thought otherwise.

We take a positive view of the measures proposed in the report to reinforce our 3R competence, including the proposal for a 3R Competence Centre. If, however, this is to be more than yet another PR exercise aimed at concerned citizens and tax payers – as might be supposed on the basis of the weak results achieved by the 3R Foundation, which has been run for decades by the Confederation and the industry – some decisions that are brave but necessary for Switzerland as a research and science location must now be taken:

1. The national 3R Competence Centre must be funded with enough money and must be networked with the universities, industry and the Cantons. It should serve these groups and facilitate their animal protection activities. In the opinion of SAP, the planned Competence Centre could also be set up as a central Federal animal experiment approval centre, in order to take pressure off the Cantons. The Cantonal veterinary authorities could then concentrate on their core competencies and the large number of Cantonal animal testing commissions would no longer be required. In the current model, involving the Cantonal animal testing commissions, the relationship between cost and revenue is poor, including in relation to animal protection. However, the Competence Centre is also expected to serve society by keeping the public regularly informed about efforts and developments in relation to 3R.
2. In the opinion of SAP, Reduce and Refine are part and parcel of good laboratory practice, and are a quite natural aspect of research activities at university and in industry. The universities in particular are given a good CHF 100 million from the state coffers for research on animals, and still enjoy an extremely broad range of opportunities for research into Reduce and Refine and for the creation, procurement and implementation of the appropriate expertise in their own research with animals, potentially by installing the university's own 3R Animal Welfare Officer.
3. Alternative methods are often highly innovative and incorporate a major scientific and economic potential. Quite apart from the fundamental ideological question of "Animal testing: Yes or No?" there is probably broad agreement that investment in this technology of the future helps to advance Switzerland's position as a research and business location. It would be fatal to leave this field to the Americans and the EU. This would be a profitable area for joint investment by the Confederation and the Cantons, as well as a wide variety of business sectors, in university professorships and research institutions concentrating on different approaches and areas of activity that are as application-oriented as possible. The justification for any such state support would include Article 22 of the law on animal protection and would undoubtedly be appreciated by tax payers.
4. The use of national research funds for stressful experiments with and upon animals must periodically be examined for significance and usefulness. The same requirement applies for projects intended for the promotion of alternative methods (acquisition of knowledge, benefits for humanity, for the animal kingdom and for the environment, implementation in practice (product development, economic success), etc.).
5. The Swiss Federation should work more closely with the OECD and other accrediting authorities so that the validation and implementation of alternatives can go ahead more quickly.

Presentations of the 10th Conference on Animal Testing

The Quality and Validity of Animal Experiments



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Introduction

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Two recent studies have pointed to the inadequate quality and validity of many animal experiments at Swiss universities and technical universities. Similar studies in other countries have come to the same conclusions. Do animal experiments have a scientific problem, as well as ethical and animal welfare concerns? This important issue is the focus of this year's 10th Annual SAP Conference on Animal Testing.

If we cannot be certain of the scientific quality of animal experiments, we must also question the validity of these experiments and the knowledge gained from them. What are the consequences of this fact, not only for researchers at universities and technical universities, but also for politicians and the authorities?

Is it not high time for us to put the research model based on "animal experiments" behind us, and to use the annual funding raised by taxation (far in excess of CHF 100 million) more sensibly – i.e. in the 3R Principles of Replacement, Reduction and Refinement – and, in particular, in the promotion and implementation of alternative methods?

It is a fact that the 3R Principles have not become as well-established as lawmakers intended in their 1993 law on animal protection. Even though alternative methods have a proven economic and scientific potential, this has rarely been used to any extent at all in Switzerland so far.

Today, the factual and emotional aspects of this topic will be discussed by our authoritative presenters from this country and abroad.

The scientific quality of animal testing – insights and measures

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In the wake of the "reproducibility crisis" in the biomedical sciences, questions are also being asked about the knowledge gained from animal experiments, and thus about their ethical reliability and legal legitimacy. The quality of animal experiments in Switzerland has been examined within the framework of two scientific studies using the criteria of good scientific practice, on behalf of the Swiss Federal Food Safety and Veterinary Office FSVO (Vogt et al., 2016; Reichlin et al., 2016) and with a view to taking potential measures to avoid any loss of confidence.

As a first step in this investigation, all the applications for animal experiments submitted in 2008, 2010 and 2012 (n = 1277) and a random sample of the scientific publications based upon them (n = 50) were checked for any mention of seven criteria of good scientific practice. These criteria include the establishment in advance of the size of the sample, the random allocation of the laboratory animals to the various test groups (randomisation), the gathering of data without any knowledge of which animals belonged to which test groups (blind trials) and a specific plan for the statistical evaluation of the data. Adherence to these criteria is a pre-condition for genuine, valid results. It became evident that specific details of adherence to scientific quality criteria were seldom provided, in either the applications to carry out the experiments or any publications. For example, we found information in less than 20% of all applications for animal experiments and publications about whether the necessary size of random testing sample had been carried out in advance, whether the animals were assigned randomly to the test groups, whether the data were gathered blind and whether they were evaluated in accordance with a specific plan (Vogt et al., 2016).

The extent to which such details – or their absence – in trials and publications can provide conclusions about the actual quality of the animal experiments is, however, disputed. For this reason, all the *e-animal experiments* were collected into a centralised information system as part of a second step, and scientists working in a responsible position on current animal experiments (n = 1891) were asked as part of an online surveyabout which of these criteria they actually adhered to within the framework of the animal experiments carried out by them, and what information about them they had provided in their most recent scientific publication. Close to 30% of the scientists approached took part in this survey and about (16%) of these completed the online questionnaire in full, and were thus included in the evaluation. A representative sample was used for the data distribution.

Based on the results of the online survey, adherence to scientific quality criteria was significantly greater that had been indicated via the experiment applications and publications. For example, 86% of the participants indicated that the animals were distributed to the trial groups in a randomised manner, but only 44% indicated that they had mentioned this explicitly in their most recent publication. The same is true for the other criteria, e.g. for the calculation of the size of sample (69% indicated that they had done this, but only 18% indicated that they had mentioned this in their most recent publication) or for the "blind" acquisition of data (47% compared with 27%).

One the one hand, these results indicate clearly that data collection from details provided in animal experiment applications or publications can be assumed to underestimate actual adherence to the criteria of good scientific practice. On the other hand, they also indicate that the researchers overestimate the quality of the way they carry out their research. For example, 44% of the researchers stated that they had provided specific information about the randomisation of the laboratory animals, but the equivalent information was actually only found in 17% of the publications under investigation. Furthermore, both the results of the online survey and the accompanying interviews with selected researchers point to a lack of awareness of the problem and insufficient knowledge of scientific quality assurance methods (Reichlin et al., 2016).

Adherence to scientific quality criteria is, like adherence to the 3R principles, a fundamental condition for the ethical justification of animal experiments within the parameters of a balance of interests (Würbel, 2017). According to the Animal Protection Act, stressful animal experiments must be reduced to the unavoidable minimum. This also includes the requirement that animal experiments must deliver significant results. Adherence to the investigation criteria of good scientific practice is therefore also a basic requirement from the legal standpoint for the approval of applications to carry out animal experiments. Within the framework of current approval practice, however, the applicant is largely simply trusted to adhere to these criteria. The results of both the studies undertaken here indicate that this trust is unlikely to be justified in many instances. Education and training in the methods of good scientific practice and scientific integrity should be extended in order to avert the risk of a loss of confidence and to reinforce the responsible institutions in their tasks. Furthermore, the process of approval for animal experiments should be checked for any potential to improve and reformed accordingly.

References

Reichlin, T. S., Vogt, L. and Würbel, H. (2016). The researchers' view – Survey on the design, conduct, and reporting of in vivo research. *PLoS One 11*, e0165999. doi:10.1371/journal.pone.0165999

Vogt, L., Reichlin, T. S., Nathues, C. and Würbel, H. (2016). Authorization of animal experiments in Switzerland is based on confidence rather than evidence of scientific rigor. *PLoS Biol 14*, e2000598. doi:10.1371/journal.pbio.2000598

Würbel, H. (2017). More than 3Rs: The importance of scientific validity for harm-benefit analysis of animal research. *Lab Anim 46*, 164-166. doi:10.1038/laban.1220

Systematic reviews of animal experiments reveal limitations for research and clinical utility

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Widespread reliance on animal models during preclinical research and toxicity testing assumes their reasonable predictivity for human outcomes. However, of 20 published systematic reviews examining human clinical utility located during a comprehensive literature search, animal models demonstrated significant potential to contribute toward clinical interventions in only two cases, one of which was contentious.

Included were experiments expected by ethics committees to lead to medical advances, highly-cited experiments published in major journals, and chimpanzee experiments – the species most generally predictive of human outcomes. Seven additional reviews failed to demonstrate utility in reliably predicting human toxicological outcomes such as carcinogenicity and teratogenicity. Results in animal models were frequently equivocal, or inconsistent with human outcomes. Consequently, animal data may not be considered generally useful for these purposes.

Regulatory acceptance of non-animal models is normally conditional on formal scientific validation. In contrast, animal models are simply assumed to be predictive of human outcomes. These results demonstrate the invalidity of such assumptions. The poor human clinical and toxicological utility of animal models, combined with their generally substantial animal welfare and economic costs, demand greater rigour within animal studies, and justify a ban on animal models lacking scientific data clearly establishing their human predictivity or utility.

Computerised neuroscience as an alternative to animal models for electrostimulation treatments for the brain

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In the scientific literature, we often read that more animal experiments must be carried out, because it would be impossible to understand the mechanisms responsible for neurodegenerative diseases in the absence of such experiments on animals, or in order that we can understand the mechanisms responsible for the diseases, to improve human health, or to develop new methods of medical treatment. In two articles that appeared recently (Verdier et al., 2015; Benazzouz et al., 2016), the scientists mentioned the vital role played by experiments on non-human primates in the discovery of a surgical intervention called DBS (*Deep Brain Stimulation*), in which this DBS is used to treat the symptoms of Parkinson's Disease. The choice of DBS as an example was not a coincidence, because this treatment approach is currently very often used for Parkinson's patients. According to Greek and Hansen (2012), this treatment is often quoted as an "example of the importance of animal testing, which justifies the continuation of this work". According to the Parkinson's Disease Foundation, more than 10 million people suffer from this disease worldwide, and approx. 150,000 of these are treated with DBS (Coenen et al., 2015).

Is it really necessary or reasonable to reach for animal experiments in the development and testing of cerebral neuro-stimulation methods, with which we can treat dysfunctions of the human brain in future? This presentation will tackle this question in three sections.

1 Historical, logical and mechanistic perspectives

From a *historical viewpoint* the articles by Verdier et al. (2015) and Benazzouz et al. (2016), in which several animal experiments were used, were penned by scientists who are among the leading figures for animal experiments. This leads to a bias in the way they select and analyse historical facts. For example, Benazzouz et al. (2016) mention the crucial role of the experiments undertaken on non-human primates in the "discovery" of DBS in a deep nucleus, which we call the *nucleus subthalamicus*. In addition, it is claimed that it would have been impossible to contrive the DBS of this nucleus in the absence of animal experiments. However, the authors forget to mention that some years before the advent of this DBS *nucleus subthalamicus*, it proved possible to undertake DBS in a different nucleus of the thalamus, the *nucleus ventralis intermedius* (VIM). In fact, Benabid et al. (1987) carried out his first DBS on patients in 1987, and supported this work on studies such as those undertaken with microelectrodes on humans in the 1960s by neurophysiologists such as Albe-Fessard et al. (1963) in France and Jasper (1966) in Canada. The reconstitution of the facts is an essential procedure in the reporting of previous procedures, affecting the selection and interpretation of the received elements. Scientists who claim that animal experiments were necessary for the invention of DBS do not provide the evidence to support this claim. The truth is that a partial description of the historical events provides a biased description and thus inevitably leads to a lack of understanding; this does not advance the discussion at all, because each party then defends his or her position (see Benabid et al., 2015; Bailey, 2015; Bailey and Taylor, 2016).

From a *logical viewpoint*, the fact that new findings are gained from animal experiments is not in question here. Many lessons have been learned from them, especially in neuro-anatomy, but Greek and Hansen (2012) point out that "some of the findings cannot be applied to humans, and some were learned independently of animal experiments" (P.3). It is clearly pointed out that new knowledge was facilitated by animal experiments, especially in the 19th century – but do we have to infer that animal experiments are still necessary in this area in the 21st century? Not necessarily, since we would be ignoring the theoretical and technological transformations that we have experienced over the past 25 years. Furthermore, this type of argument would fail to recognise the discoveries made in the area of cerebral stimulation – and elsewhere – in part as a result of pure coincidence, but also from a stroke of good fortune (Little et al., 2013; Benabid and Torres, 2012). Finally, in the third part, we will see from the work of Frank (2005) that we also need to consider the problem in an economic context.

From a *mechanistic point* of view, DBS seems to work by stimulating local myelinated axons that belong to a large neuronal network and modulating the oscillations that are excessively synchronised in the beta wave frequency band (i.e. at 13-30 Hz), using a mechanism that has not yet been fully explained; as Wichmann and DeLong modestly stated in 2016, "It is unlikely that the DBS will return normal function to people with motor defects. It looks more likely that the DBS replaces an abnormal activity in the basal ganglia with a slightly more acceptable activity, and that this contributes to the restoration of a certain functionality in the downstream neuronal networks" (Wichmann and DeLong, 2016). Neither is the argument very convincing that we would not have developed DBS if we had not previously undertaken animal experiments in order to understand the mechanisms involved in DBS. In reality, we still do not yet know everything about exactly how DBS works. For example, Muñoz et al. (2016) described how the "preclinical models based

on neurotoxins acted as valuable tools in our understanding of certain mechanisms. On the other hand, they do not seem to reflect what is happening in Parkinson's disease and are therefore unhelpful for the development of new treatments" (P.1).

In fact, the experiments carried out on animals such as rodents or apes, whether they are of a toxic, cellular or genetic nature, fail to match the slow, progressive, degenerative form of Parkinson's disease in humans (van der Worp et al., 2010), nor the observed variations in the clinical phenotypes, nor the fluctuating nature of the symptoms (Blesa and Prezedborski, 2014). As a result, it is difficult to imagine how the instigation of more animal experiments could represent an appropriate strategy for the conception and development of treatment methods using cerebral stimulation in Parkinson's syndrome. The argument is frequently put forward that similar neuronal networks exist in humans and animals; this is not false, but there is no guarantee that these networks are controlled in the same way in both species (Molnár et al., 2016).

Furthermore, the research work carried out by Goulas et al. (2014), in which structural inter-regional connections in the brains of macaques and humans are compared, demonstrates that – beyond the similarities in the connectivity of both species – there are a number of rearrangements in the form of reconnections or expansions that have appeared on the macroscopic level in the course of the evolution of primates, and that the unique properties of the human brain are concealed behind these changes. In conclusion of this first section, we can state that any demand for more animal experiments in the area of cerebral stimulation would not be based on solid facts.

2 Why is computational neuroscience a credible alternative?

In view of the current context, now seems to be the correct time to bring together the latest advances in technology, theory and IT engineering.

The *first technological advance* comes from the cerebral imaging field and from biomedical technology. Hickey and Stacy (2016) talk about new stimulation electrodes, the "adaptive" stimulation (in the closed control cycle) of a DBS controlled by an imaging process, imaging in real time interventions and imaging in humans using a diffusion tensor *in vivo*. This leap in technology allows us to visualise and validate sections of the neuronal networks that contribute to human cerebral disorders, and they make it possible to clarify the details of any overlapping or redundancies that may occur in these networks (Weingarten et al., 2015).

The *second technological advance* comes from the area of IT: the speed and capacity of computing increases exponentially and doubles every two years or so, as Moore's Law predicted back in 1965. These new options can be applied to ever-larger databases, and combined with exponentially larger storage capacities and falling storage costs. This drove Van Horn and Toga (2014, p.2) to write that the imaging processes applied to the human nervous system (*neuroimaging*) would belong in the realm of "Big Data" in future.

The third important advance comes from the area of maths and physics. It has become clear that brain rhythms are omnipresent (Thut et al., 2013). We have known about these rhythms for about 100 years (Little and Bestmann, 2015), and they can be described in terms of their frequency, their amplitude and their phase. As a result, they can be modelled (and this is important information). For example, Jirsa et al. (2010) modelled the brain as a complete entity made up of complex dynamic networks and investigated the effects of (virtual) changes in these networks in order to understand how cerebral disorders come about and how we could re-establish normal functions and "repair" these disorders through the influence of the network effect.

The combination of these technological, theoretical and IT advances open up new opportunities for the modelling of the neuronal networks of human patients. Computer-supported modelling undoubtedly offers the *formalism* with which extremely complex problems can be "simplified" and with which we can, for example, investigate how functional units connected to neuronal networks react when they experience an exogenous stimulation, or we can investigate how, if at all, the stimulation of a specific network attacks the behaviour of a healthy or sick individual (Little and Bestmann, 2015). In addition, neuronal networks are now described on more spatial, chronological and topological levels (Betzel and Bessett, 2016).

These models also offer a *global* (or holistic) *approach* with regard to the results produced by the system in terms of the dynamic obtained under different conditions (Wang et al., 2015, p. 192). On the one hand, they allow us to understand the circumstances under which a healthy brain circuit becomes diseased; on the other hand, they also allow the values of the control parameters that need to be changed to be determined in order that we can return the system into a non-pathological zone. They provide us with information about the way in which the desynchronization of the oscillation in the neuronal networks becomes visible, i.e. how the cerebral disorders correspond to a deregulation of the oscillations and how a therapeutic cerebral stimulation can modulate these oscillations and bring them into a "healthy" range (Modolo et al., 2011).

Finally, these models make it possible to carry out *virtual experiments*. They allow a limited number of convincing hypotheses to be tested in real, physical experiments, so that we can then optimise and reduce their number. "These days, the improvement in the neuro-stimulation hardware combined with the control provided by the reliable biophysical models of the activity of the cerebral tissues must necessarily convince us that the time has come to concentrate our efforts on research in humans in order to yield new treatments for neuro-modulation in Parkinson's disease". (Modolo et al., 2015, p. 2).

In conclusion, we can establish from this second section that there is a powerful discrepancy between the human cerebral network and a virtual, computer-controlled network, but that this discrepancy is becoming ever easier to bridge, because modelling has become more realistic on both the biological and neurophysiological levels. These types of models need to be tested on humans so that the fluctuating condition of the brain of each

patient and the development of that patient's illness can be taken into account; animal experiments do not allow this to happen because they are less adaptable (because they are of a cellular, toxic or genetic nature) and they depend upon on the selected animal species. What are the aspects that currently impede the transition from animal experiments to experiments using *computational neuroscience* in the area of neuro-modelling?

3 Discussion and conclusions

The economist Joshua Frank (2005) describes these obstacles as a form of lock-in effect, which can be of an institutional or behaviour-based nature. Put succinctly, this barrier is associated with a decision, and corresponds to the prevailing position of a paradigm, a technology, a method or a product – not on the basis of their low intrinsic costs or good performance, but because they occupy a dominating position or demonstrate an attractive return.

The first barrier is based on a lack of understanding of the computer models used in the neurology sector – because these models are still new – while animal experiments are very familiar. Bonate (2014, p. 417) wrote that "most people do not know what a computer model is, how it works, what makes it a good model or how it can be evaluated". In order to understand a computer model, it is necessary to have a certain knowledge of mathematics, IT, statistics and physics and to have access to clear educational explanations. It is important for the strengths and limitations of a model to be explained in simple words in order to clarify how the different components of a model represent its proximity to reality, and to explain which aspects of this reality are not taken into account because of the simplifying hypotheses in the model (Teufel and Fletcher).

An educational, cooperative approach means accepting a change of culture, changing your mind – and being aware that logic and reason has very little to do with change, because most opinions rest upon convictions, not facts (Bonate, 2014). As Duhigg (2013) describes this situation, it is important that we do not underestimate the power of habits that are firmly fixed in our brains and account for almost half of our decisions. According to Frank (2005) this is a behaviour-based barrier. "Even though scientists already possess a cultural inheritance, their long years of study and training lead these scientists to create a second cultural inheritance, in which they accept that animal experiments are ethically acceptable and represent them as the least of the necessary evils.

A scientist in a particular area, carrying out animal experiments, mainly comes into contact with the results of studies undertaken by other researchers, who have also carried out experiments on animals. The findings of this scientist will undoubtedly reinforce his belief in this type of research. Researchers who work with animal experiments also find it easier to quote other scientists who carry out research on animals, than to quote the results of studies based on alternative methods. This could explain an iterative strengthening of the individual's own convictions" (Frank, 2005, p. 562-563). The system therefore incorporates an inertia, which – depending on the author – is more strongly marked than elsewhere in the research community where animal experiments are used. Why is this? Because animal experiments have become a kind of tradition, based around certain bodies, such as ethics boards (who are not always informed about the poor reliability of the animal experiments) and also because studies involving animals can offer the enterprise some protection against legal problems.

A second barrier is of an economic and financial type. Changing the DBS economic model and changing over from animal experiments to *computational neuroscience* for tests on methods of treatment for the future means a radical change and a potential, significant loss of profits for the extremely lucrative medical technology market. DBS is reimbursed as a surgical intervention by the health insurance schemes of several countries. The cost of bilateral DBS amounts to 70,000 to 100,000 US dollars. For the 150,000 patients who have undergone surgery so far, this results in an overall total of 15 billion USD. The loss of this profit – even temporarily – represents an unacceptable risk for large corporations, who generally prefer to leave the financing of the *proof of concept* studies to the public institutions and then buy out start-ups at a later stage, when the anticipated profits are secure. As a result, DBS based on animal experiments may continue to dominate the market, since it continues to generate profits or a certain yield, while keeping the risks within limits. These institutional barriers are also acceptable to the companies who breed animals, those who carry out animal experiments, those who supply the equipment and the state-run facilities that finance this research, and the various lobbies that support animal experiments (Frank, 2005). Added to this are the financial aspects, which are sometimes expressed in conflicts of interest, because certain scientists act as paid consultants to the medical technology companies who also finance their research. This understanding between research and financial donors is not a problem in itself, but it can become problematic if conflicts of interest decelerate innovation and rob patients of more efficient, less invasive treatments at an earlier stage.

The final barrier actually comes from the many administrative, legal and practical political constraints. The process involved in CE marking for medical products is not just technically challenging, it is also protracted and costly. Some European directives are currently being revised and modified, but specific directives and ethical constraints also affect each European country. Alim Louis Benabid, a pioneer in DBS, recently said in an interview that it would undoubtedly be difficult to test DBS on humans in the current context in the face of the many complex constraints.

To summarise the above, I have attempted to demonstrate that realistic neurological computer models could represent a viable, dependable alternative to animal experiments in the development of innovative treatment methods for Parkinson's Disease that are based on electrical cerebral stimulation on the biological and neurophysiological levels. I have also tried to analyse the institutional and behaviour-based barriers that slow down the transition from animal experiments to medical *computational neuroscience*. These barriers will be overcome when the ac-

ademic, industrial and political circles become aware of them and decide to act by providing the alternative approaches with greater support and visibility. This will make human medicine stronger and more personal.

References

Albe Fessard, D., Arfel, G., Guiot, G. et al. (1963). Characteristic electric activities of some cerebral structures in man. *Ann Chir 17*, 1185-1214.

Bailey, J. and Taylor, K. (2016). Nonhuman primates in neuroscience research: Scientifically unnecessary. *Altern Lab Anim 44*, 43-69.

Bailey, J. (2015). Letter to the editor. *Altern Lab Anim 43*, 2-3.

Benabid, A. L., Pollak, P., Louveau, A. et al. (1987). Combined (thalamotomy and stimulation) stereotactic surgery of the Vim thalamic nucleus for bilateral Parkinson's disease. *Appl Neurophysiol 50*, 344-346.

Benabid, A. L. and Torres, N. (2012). New targets for DBS. Parkinsonism *Relat Disord 18*, S21-S23. doi:10.1016/S1353-8020(11)70009-8

Benabid, A., Delong, M. R. and Hariz, M. I. (2015). Letter to the editor. *Altern Lab Anim 43*, 427-428.

Benazzouz, A., Gross, C. and Bioulac, B. (2016). Non-human primate: An essential building brick in the discovery of the subthalamic deep brain stimulation therapy. *Front Aging Neurosci 7*, 252. doi:10.3389/fnagi.2015.00252

Betzel, R. F. and Bassett, D. S. (2016). Multi-scale brain networks. *NeuroImage 160*, 73-83. doi:10.1016/j.neuroimage.2016.11.006

Blesa, J. and Przedborski, S. (2014). Parkinson's disease: Animal models and dopaminergic cell vulnerability. *Front Neuroanat 8*, 1-12. doi:10.3389/fnana.2014.00155

Bonate, P. L. (2014). What happened to the modeling and simulation revolution? *Clin Pharmacol Ther 96*, 416-417. doi:10.1038/clpt.2014.123

Coenen, V. A., Amtage, F., Volkmann, J. and Schläpfer, T. E. (2015). Deep brain stimulation in neurological and psychiatric disorders. *Dtsch Arztebl Int 112*, 519-526. doi:10.3238/arztebl.2015.0519

Duhigg, C. (2013). *The Power of Habit* (371pp). London, UK: Random House Books.

Frank, J. (2005). Technological lock-in, positive institutional feedback, and research on laboratory animals. *Structural Change and Economic Dynamics 16*, 557-575. doi:10.1016/j.strueco.2004.11.001

Goulas, A., Bastiani, M., Bezgin, G. et al. (2014). Comparative analysis of the macroscale structural connectivity in the macaque and human brain. *PLoS Comput Biol 10*, e1003529. doi:10.1371/journal.pcbi.1003529

Greek, R. and Hansen, L. A. (2012). The development of deep brain stimulation for movement disorders. *J Clinic Res Bioeth 3*, 1000137. doi:10.4172/2155-9627.1000137

Hickey, P. and Stacy, M. (2016). Deep brain stimulation: A paradigm shifting approach to treat Parkinson's disease. *Front Neurosci 10*, 173. doi:10.3389/fnins.2016.00173

Jasper, H. H. (1966). Recording from microelectrodes in stereotactic surgery for Parkinson's disease. *J Neurosurg 24*, 219-221.

Jirsa, V. K., Sporns, O., Breakspear, M. et al. (2010). Towards the virtual brain: Network modeling of the intact and the damaged brain. *Arch Ital Biol 148*, 189-205.

Little, S., Pogosyan, A., Neal, S. et al. (2013). Adaptive deep brain stimulation in advanced Parkinson disease. *Ann Neurol 74*, 449-457. doi:10.1002/ana.23951

Little, S. and Bestmann, S. (2015). Computational neurostimulation for Parkinson's disease. *Prog Brain Res 222*, 163-190. doi:10.1016/bs.pbr.2015.09.002

Modolo, J., Legros, A. and Beuter, A. (2015). The next move in neuromodulation therapy: A question of timing. *Front Comput Neurosci 8*, 162. doi:10.3389/ fncom.2014.00162

Molnár, G., Rózsa., M., Baka., J. et al. (2016). Human pyramidal to interneuron synapses are mediated by multi-vesicular release and multiple docked vesicles. *eLife 5*, e18167. doi:10.7554/eLife.18167.001

Muñoz, P., Paris, I. and Segura-Aguilar, J. (2016). Commentary: Evaluation of models of Parkinson's disease. *Front Neurosci 10*, 1-3. doi:10.3389/fnins.2016.00161

Teufel, C. and Fletcher, P. C. (2016). The promises and pitfalls of applying computational models to neurological and psychiatric disorders. *Brain 139*, 2600-2608. doi:10.1093/brain/aww209

Thut, G., Miniussi, C. and Gross, J. (2012). The functional importance of rhythmic activity in the brain. *Curr Biol 22*, R658-663. doi:10.1016/j.cub.2012.06.061

van der Worp, H. B., Howells, D. W., Sena, E. S. et al. (2010). Can animal models of disease reliably inform human studies? *Plos Med 7*, e1000245. doi:10.1371/journal.pmed.1000245

Van Horn, J. D. and Toga, A. W. (2014). Human neuroimaging as a "Big Data" science. *Brain Imaging Behav 8*, 323-331. doi:10.1007/s11682-013-9255-y

Verdier, J.-M., Acquatella, I., Lautier, C. et al. (2015). Lessons from the analysis of non human primates for understanding human aging and neurodegenerative diseases. *Front Neurosci 9*, 1-6. doi:10.3389/fnins.2015.00064

Wang, Y., Hutchings, F. and Kaiser, M. (2015). Computational modeling of neurostimulation in brain diseases. *Prog Brain Res 222*, 191-228. doi:10.1016/bs.pbr.2015.06.012

Weingarten, C. P., Sundman, M. H., Hickey, P. and Chen, N. K. (2015). Neuroimaging of Parkinson's disease: Expanding views. *Neurosci Biobehav Rev 59*, 16-52. doi:10.1016/j.neubiorev.2015.09.007

Wichmann, T. and DeLong, M. R. (2016). Deep brain stimulation for movement disorders of basal ganglia origin: Restoring function or functionality? *Neurotherapeutics 13*, 264-283. doi:10.1007/s13311-016-0426-6

The licensing procedure for animal experiments in Switzerland

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Definition of animal experiments (Art. 3 of the Animal Welfare Act)

Any measure in which a live animal is used with the aim of
- Testing a scientific assumption
- Determining the effect of a particular measure in the animal
- Testing a substance
- Obtaining or testing cells, organs or bodily fluids
- Obtaining or replicating organisms alien to the species in question
- Assisting in teaching or training

Dilemma

We all want
- Effective drugs
- Safe chemicals and effective agents

Nobody wants
- Suffering, anxiety, stress or pain in animals

Nobody enjoys carrying out animal experiments!
Duty of approval for every animal experiment and for the husbandry of every laboratory animal

Tab. 1: Types of animal research and goals

	Type of research	Goal (Art.3)
66%	Basic research	• Testing a scientific assumption • Obtaining or testing cells, organs or bodily fluids • Obtaining or replicating organisms alien to the species in question
31%	Clinical studies	• Testing a substance (proof and evaluation of the relationship between dosage and effect) • Obtaining or testing cells, organs or bodily fluids
	Regulatory animal experiments for quality assurance purposes	• Testing a substance (proof and evaluation of the relationship between dose and effect: drugs, vaccines, chemicals)
3%	Experimental behavioral biology	• For teaching, education and training purposes
	Education/teaching (universities, secondary schools, laboratories)	• For teaching, education and training purposes

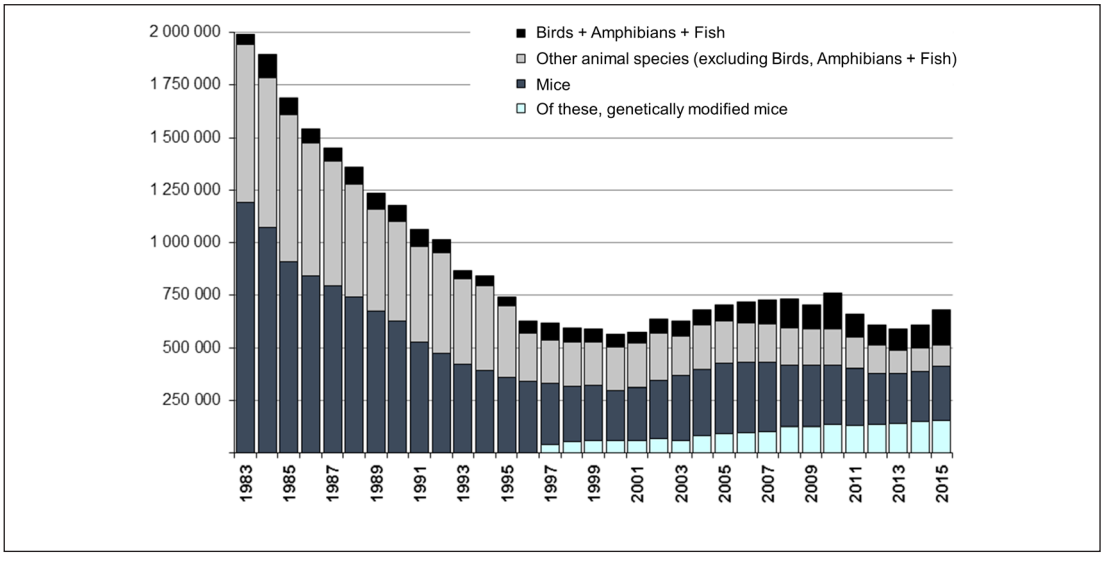

Fig. 1: The changes in animal experiments

Alternatives (Replace)

- Alternative methods must be used where these are available
- The research and development of alternative methods is complex and calls for major funding over an extended period
- We do not expect research that is free of animal experiments to be possible in the short to medium term

Reduce and Refine

Until we can do without animal experiments, I am committed to ensuring that:

- As few animals as possible have to be used for animal experiments
- The animals used in animal experiments are spared any unnecessary stress in the way they are kept, both during and after the animal experiment

The purpose of the approval process and the examination of each individual case is to ensure the above.

Balance of interests and level of severity

- *Balance of interests:* the expected advantages are greater than the stress caused to the animals
- *Unavoidable extent:* Stressful animal experiments must only be carried out if they are absolutely necessary, and must be as gentle as possible
- *4 levels of severity* (0 to 3)
  → 0: 43% no stress
  → 1: 34% slight stress
  → 2: 21% medium stress
  → 3: 2% severe stress

Tab. 2: Levels of severity

	Examples
0	Improvement in the husbandry systems for chickens Video recording for studies on the dehorning of kid goats Removal of organs for *in vitro* experiments
1	Experiments carried out under full anaesthetic, where the animal is euthanised while still under anaesthetic Vaccination, euthanasia and collection of blood samples to prove the efficacy of vaccines
2	Research into diabetes, cancer, arthritis and hip replacements
3	Research into chronic pain, heart attack, stroke and autoimmune diseases

Federal Council Report (1 July 2015) (In fulfilment of Postulate 12.3660)

The Federal Council acknowledges that action is required in the following areas:

- Strengthening of 3R research
  - Creation of a national 3R Competence Centre (3RCC)
  - Initiation of research and validation of the 3R methods (→3RCC)
- Expansion of education, training and ongoing training for researchers in the area of 3R (→3RCC and universities)
- Publication of information relevant to 3R. Publication of negative results of experiments (→3RCC)
- Creation of the Animal Welfare Officer function in the Animal Welfare Ordinance (Revision of the Animal Welfare Act)
- Design of experiments: optimisation of the flow of information

Applications and Approvals

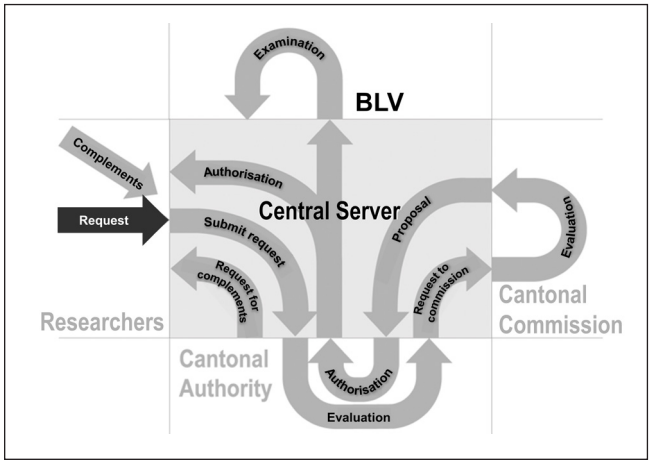

Fig. 2: Processing of researchers' requests by the FSVO (BLV), cantonal authorities and cantonal commission

Federal Supervisory Authority

Examination of Form-A

- Unavoidable extent
- Anaesthesia and/or other analgesia
- Details of measures to alleviate stress. Termination criteria
- Monitoring of the animal's welfare
- The fate of the animal after the experiment, method of euthanasia
- Balance of interests
- ….

Findings and areas for action

Internal validity: design and execution of experiment

- Animal Welfare Officer for animal experiments (AWO) in institutions where animal experiments are carried out
- Function: provide proactive advice from the design of the experiment onwards

→ Embedding of the Animal Welfare Officer (AWO) in the TSchV (Revision 2017)

Tab. 3: The Würbel Study and the application for animal testing (Form-A)

Form-A	**No. …… (To be completed by the approval office)**

Application for animal experiments Article 18 of the Animal Protection Act (TSchG), Article 141 of the Animal Experiment Ordinance (TVV)	
5	**DETAILS OF THE METHOD (Description and comments on Clauses 51-58)**
51.1	Overview of the project (Project organisation, overview of method/name of animal model, experimental procedure/ flow chart, biometric planning) (Details of method under Clause 54)
51.2	Reason for the choice of method or model, providing details of particular aspects/advantages (Art 137, Para 3 of the Animal Protection Ordinance, TSchV)
51.3	Reason for the choice of animal species and (if relevant) for the use of animals not bred for experimental purposes
54.3	Number of animals per experiment/series of experiments: Number of groups (Incl. all variables, e.g. doses, duration, checks and details of scheduling of experiments in accordance with Art 137, Para 4Bstc of the TSchV) and number of animals per line, group, gender of the animal
54.4	Reason for the anticipated number of animals per experiment/series, including the statistical handling of data (Art 137, Para 4Bstc of the TSchV)
55	Evaluation of the method (Art 137, Para 3 of the TSchV)

Tab. 4: Application clauses involved in the Würbel study

Würbel study		**Form-A, Clause (s)**
Constructive validity	Scientific validity	51.1, 51.2, 51.3, 55
Internal validity	Design and execution of experiment	54.1-55.4
External validity	Comparison with other research group in different laboratories	Will not be checked with the application for animal experiments

Findings and areas for action

Internal validity: design and execution of experiment

- Curriculum for the biomedical courses: from as early as the Bachelor level, give more weight to experimental design, statistical significance and 3R principles
- The swissuniversities Conference of Rectors supports this teaching position through the 3R Competence Centre
- Development: Cantonal animal experiment committees, enforcement agencies and Animal Welfare Officers

→ Evaluation of application documents, unavoidable extent, suitability of the statistical assessment, etc.

Findings and areas for action

Constructive validity: scientific validity

- Research foundations (e.g. SNSF) check the "state of the art" of research projects
- Lack of highly specific experimental skills in the animal experiment committees and approval authorities

→ Use the expert knowledge of the research foundations as decision-making bases

→ *Optimisation of exchange of information between research foundations and approval authorities*

Summary

1. Creation of a national 3R Competence Centre
2. Embedding of the Animal Welfare Officer (AWO) in the TSchV (Revision 2017)
3. Further intensification in the training and ongoing training of researchers, members of the animal experiment committees and approval authorities
4. Experiment design and 3R education for students in the Life Sciences faculties at the Bachelor level
5. Optimisation of exchange of information between research foundations and approval authorities

"Quality inadequate" – the perspective of a member of an animal testing committee

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Switzerland is proud of its position in the world of research; by its own assessment, it is a location where world-beating research is being carried out. It also celebrates its animal protection law and regards that too as a world leader. There is a strict licensing procedure in place in relation to animal experiments. On looking closer, however, it transpires that this area involves an immense administrative cost and is understandably regarded as a burden by researchers, but from an animal protection and scientific viewpoint, it presents barely any barrier to some questionable research projects. This situation is legally unsustainable, as illustrated below.

Because animal welfare and the dignity of animals are constitutional rights, and therefore rank the same as fundamental rights and other national objectives, any infringement of the relevant protective interests is only permissible if this is required by an "overriding interest" in that specific individual case. Research projects involving animals are therefore subject to approval. Approval for an experiment that is associated with stress for the animals involved in that experiment is dependent on a variety of different conditions. In particular, the trial must be proportionate; a test of whether the animal experiment is suitable and absolutely necessary, or even mandatory, in order to achieve the goal of the research must therefore be carried out. The interest in the experiment and its results must therefore be weighed against the stress it causes to the animals. The experiment may only be approved if the benefit gained from the experiment clearly outweighs the damage to the wellbeing and dignity of the animal. As part of the approval proceedings, the three steps of suitability, necessity and proportionality (balance of interests) in the narrowest sense must therefore be taken into account by law – in addition to some preliminary questions, such as training for the staff, infrastructure and general conditions. The current system fails at all three levels, which means that the legal requirement to link animal experiments to the "unavoidable level" will not be fulfilled.

In practice, a standard has become established on the balance of interests level in the approval process for animal experiments over the years. This is no longer subject to any serious scrutiny, even though the animal protection law requires continuous critical scrutiny and a repeated weighing up of interests. This is regardless of the fact that the relationship between the interests has undergone considerable change, to the benefit of the animals, especially in view of the confirmation of the dignity of the animal in the applicable animal protection law in 2008. Switzerland also lags far behind other countries in the meaning of "necessary" and the associated basic requirement for alternative research methods. Cantonal authorities rely on the Confederation who, in turn, expect that this subject will be tackled by the research community.

In this case, however, the focus is on the evaluation of the quality and validity of animal experiments in Switzerland. Legally speaking, we are working on the standard of the eligibility, which the approval authorities and the animal testing committee views in relation to their goal for the long term – and which is always put forward as a justification in the balance of interests – but which is not normally discussed, on purely pragmatic grounds. For example, the question of whether a frustrated mouse can really be used to investigate the causes and mechanism of depressive illnesses is never discussed. The question of suitability is – with very few exceptions – placed trustingly in the hands of the applicant. This is understandable, in the face of the veritable flood of applications. Given the current situation where about 1000 new applications are received throughout Switzerland, together with many more additional applications for revisions and supplementations per year, it is impossible to examine them all. Even with the large number of people currently dealing with this issue (in Zurich alone, there are three officers at the veterinary office and eleven members of the animal testing committee who are responsible for animal experiments; the institutions' own internal animal welfare officers are also involved in each case), it is still impossible to examine fundamental questions carefully. In addition, the research community itself only seems to ask itself these questions sporadically and inadequately. Research groups place their trust in the results they have obtained over many years and even decades, and dive into ever newer and more exciting research problems, apparently without normally questioning their models and processes in any depth.

Dozens of questions come up and need to be taken into account in regard to the appropriateness of animal models – and neither the applicants nor their teams, nor the committee, nor approval authorities are fully equipped for this task. Particularly because of the fact that an animal can only be a fragmentary aid to solve a complex problem, at best, many influences and interfering factors may have a significant influence, e.g. when the animals are handled, the environment in which they are handled, anaesthesia, analgesia, etc. Experience has shown that these are only partially considered during the experiment planning stage, and many of them are simply ignored altogether. Even Switzerland's much-vaunted cutting-edge research makes use of more or less untested established models from all around

the world, without tapping the full potential of their spirit of innovation. Meanwhile, the fact that there must be something wrong with current practice is evidenced by the feeble rates of transmissibility and reproducibility. These do not just affect the foreign competition.

I believe that the Canton of Zurich's Cantonal Committee on Animal Testing provides an important 3R / 1R (Refinement) service. Experts from various specialist sectors endeavour to refine the planning of experiments so that the stress suffered by the affected laboratory animals is minimised, without affecting the goals of the research. However, the committee fails in its efforts to evaluate the suitability and necessity for animal experiments in relation to the long-term aims of the tests – and a Cantonal Committee on Animal Testing may also simply not be able to provide this service. The two studies currently under consideration (Vogt et al., 2016; Reichlin et al., 2016) mention deficiencies but do not concern themselves with the suitability of animal models in terms of the long-term goals, such as the ongoing advancement of medical standards. On the contrary, this question touches an even more basic level, i.e. the suitability of the specific experimental concept in relation to the knowledge immediately under investigation. The deficiencies revealed by both of these studies are a problem for Zurich too. They seem to be a component of a system that has not been examined seriously for many years. From what I have observed, there are several reasons why the committee frequently fails to investigate the associated quality-relevant questions:

1. The current practice is established. It's done like this "everywhere". The animal testing committees know no other way. They are mainly made up of researchers who work in this way themselves. Outsiders without any experience of research are unaware of the critical areas.
2. Different standards apply in basic research compared with applied or "application oriented" research; the normal quality directives are often viewed with less precision, as this type of research is much more open, and less likely to be directed towards a specific goal. It seems that this area generally tolerates more freedom with regard to research creativity.
3. If appropriate statistical sample sizes are investigated, the committee is also faced with the worry that the numbers of animals may actually increase, because the samples are frequently too small. A (too) small number of animals is therefore often preferred in comparison with more solid results, which leads to a mistrust of either the anticipated research results or the statistical calculations.
4. Biomedical research (and research involving animal testing in particular) incorporates many elements of uncertainty, which would be regarded as grey areas of research. It is generally accepted that many aspects are not subject to control, despite standardisation in some selected areas.

Scientifically speaking, biomedical research is facing a crisis in current practice. Despite the deficits that were revealed years ago (see e.g. Ioannidis JPA (2005), Why Most Published Research Findings Are False, in: *PLoS Med 2(8),* e124.) this sluggish system continues to crank along in its usual fashion. Researchers regard themselves as a part of the system, and do not regard it as their responsibility to change anything fundamental. Neither the donor institutions nor the authorities nor the politicians have the confidence to evaluate the situation. Only some of the criticism with regard to flaws affects research involving animal testing while other areas of research are also affected by quality deficiencies. This is alarming enough in view of the fact that it is impossible to use the immense resources invested in this research appropriately. In the animal testing area, the identified deficiencies are particularly controversial because they involve living beings whose welfare and dignity should be protected under Swiss law. We will fail to honour this requirement if we stubbornly continue to ignore the quality problems throughout an entire sector.

The two studies both show that Switzerland is not an outstanding research location in terms of the quality of research. As a consequence, we are faced with a flood of publications with results that are extremely difficult to judge in terms of value. Meanwhile, important attainments, such as the protection of the welfare and dignity of animals, may well be put forward as a guarantee of an ethically justified orientation, but they are not consistently implemented.

Switzerland must not miss out on the transition to alternative methods

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If the quality of animal experiments is being criticised, it is high time to focus on the promotion, development and implementation of alternative methods. In the past year, two studies initiated by several parliamentary requests on my part (specialist journals PLOS Biology and PLOS ONE) have attested to the inadequate quality and validity of Swiss animal experiments. Similar studies in other countries have produced the same results. As a politician working in the area of education and science who campaigns for more funding for the education, research and innovation sector every four years, it is therefore shocking to learn that although far more than 100 million CHF flow into our Swiss universities for animal experiments every year (compared with a few 100,000 CHF for 3R every year), the scientific quality leaves much to be desired. The acquisition of knowledge resulting from practical research and the associated justification for animal experiments looks very shaky in view of these scientifically proven deficiencies. I am delighted and very grateful to SAP for organising this specialist conference to tackle these burning questions and enabling the scientific and specialist circles to open the urgently required discussion so that alternative methods, not involving animal experiments, can finally become the scientific standard. We need this move in order to safeguard Switzerland's place as an innovative research location; we must not sleep through the signs of the times – and we must provide massive support for alternatives to animal experiments, so that they can become the norm at last.

From the political point of view, the lack of scientific quality and validity of animal experiments is also sure to have consequences. One of the results of these studies must be a change of views towards intensive support for 3R and the stronger development and implementation of alternative methods. Animal-testing free technologies are often more reliable scientifically and more transferrable to humans than animal experiments. Alternative methods have been proven to be fast, economical and reliable, as well as extremely innovative (e.g. 3D Bioprinting, Multi-Organ Chips).

In addition, the scientific quality and validity of animal experiments must be regularly evaluated and analysed in view of what we now know – in the sense of constant quality assurance testing for experiments funded by tax revenue, with a regular publication of the results of these checks on quality.

Thanks to a parliamentary order, the Federal Council / Federal Food Safety and Veterinary Office FSVO joined the State Secretariat for Education, Research and Innovation SERI to push for a report (Postulate WBK 12.3660 "The future of the 3R Research Foundation and alternative methods to animal experiments") and initiated the following in 2016.

Swissuniversities (a conference of the rectors of Swiss universities) was asked to prepare a concept for a national 3R Competence Centre. This concept is now in place and incorporates the following goals (Extract from the reply from the Federal Council, dated 15 February 2017 to my Interpellation 16.4121 More consideration of alternatives to animal experiments in education):

- In future, the subject of "research with animal experiments" should be given more weight at universities, from the Bachelor level, particularly in the biomedical sciences, and the basics of the 3R principles should be rooted and taught in research and education.
- Education in the area of animal experiments should be intensified further for researchers, members of the animal testing committee and the responsible enforcement authorities (e.g. correct scientific procedure in the planning of experiments and the evaluation of criteria used to check application documents).
- The exchange of information, developments and promotional strategies in the 3R sector should be established and secured across the whole of Switzerland. In particular, the 3R Competence Centre should promote an exchange of content between researchers and research institutions on the national and international level, the various stakeholders, and connect those who are tackling 3R-research together and bring them into dialogue.
- The gaps in knowledge in all areas of 3R must be closed and research projects (and methodical ongoing developments in particular) must be pushed and promoted.
- An application for financial support for the national 3R Competence Centre in accordance with Article 15 of the Federal Law on the Promotion of Research and Innovation (SR 420.1) is due to be submitted to SERI in the first quarter of 2017. The Federal Department for Science, Education and Research is expected to decide upon the submission at the end of 2017. Support from the Confederation for the national 3R Competence Centre should be within the framework of the current funding for research, which is 3.5 million CHF for the period 2017-2020.
- The Federal Council has also anticipated that a suitable reporting process will be established for the implementation of the 3R Principles in the Swiss National Scientific Foundation's funding practices.
- The current revision of the ordinance on animal welfare envisages a regulation that any institution or laboratory un-

dertaking animal experiments should in future nominate an Animal Welfare Officer for animal experiments. The function of this officer will be to support the implementation of the animal welfare provisions and to entrench the 3R Principles through the provision of advice and proactive information at the experiment planning stage.

Up to now, the Confederation has either not used the strong scientific and economic potential of alternative methods at all, or not used them enough. There is a danger that Switzerland will be left behind in these technologies of the future, because it puts its investment and research funds one-sidedly into an out of date and extremely costly animal testing technology. At the same time, the numbers of laboratory animals are increasing continuously in the state-supported basic research carried out at the universities – (from about 150,000 animals in the year 2000 up by 172% to 409,000 animals in 2015).

In view of the demonstrable lack of scientific quality and validity in animal experiments, the final resort must now be an improvement in the promotion of alternative methods to animal experiments in Switzerland and the establishment of an innovative location for research.

As the author of a motion submitted in the spring session of 2017 (17.3240 for an innovative Swiss research location: improved promotion of alternative methods for animal experiments) I believe that a change in the law should guarantee that animal experiments will be replaced step by step by alternative methods. We should invest at least as much of our financial resources in this as we do in methods that aim to reduce the number of laboratory animals or minimise the stress to which they are subjected. In addition, periodic information is required from the Confederation about the resources invested in these three branches of research and the resulting progress achieved.

From micro-tissues to micro-physiological systems: Opportunities to reduce the number of animal experiments

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By now, it is impossible to imagine the absence of *cell-based tests* in the research and development of pharmaceutical drugs, as they provide information about the *in-vivo* reaction to biological and chemical preparations. Cells are used as a testing system throughout the entire development chain: (i) Target validation, (ii) Allocations to the primary and secondary screening process, (iii) Optimisation of lead structures and (iv) Toxicological profiling. The current standard process technology is based on monolayer culture of cells from mammals – primary cells or cell lines – in plastic trays. However, the cells must retain a structure that corresponds as precisely as possible to the *in-vivo* cell functionality of animals or humans in order that the maximum benefit for the minimisation of risk from pharmaceutical drugs can be gained from the *in-vitro* cell cultures.

Techniques that enable direct cell/cell communication and communication between the tissues are of great importance in order to increase the value of *in-vitro* models. *Advances in 3D cell culture models gain momentum constantly, since the development of new treatments are costly in terms of time and money, and any reduction in the risk associated with pharmaceutical drugs is of great benefit*. Scalable tissue engineering strategies that are compatible with automation are used for this reason, and in order to improve the predictive power of cell-based tests still more. The more predictable the patient's reaction to the pharmaceutical drugs with the help of *in-vitro* models, the fewer animals will be needed to profile the preparation. 3D cell culture techniques combined with micro-physiological body-on-a-chip stems have the potential to allow the research and development of pharmaceutical drugs to rely fully and completely on human-based concepts.

Magnetic blood purification: From concept to application

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The direct removal of pathogenic compounds from the blood is an attractive proposition for the treatment of diseases such as blood poisoning and autoimmune diseases. A blood purification process that is based on magnetic separation is particularly useful for the removal of compounds that have a high molecular weight, which cannot be removed to a sufficient extent by conventional blood purification systems (e.g. dialysis and haemoadsorption) (Hermann et al., 2013a). Despite promising results *in-vitro* and *in-vivo* it is not easy to apply one of these processes in hospital (Herrmann et al., 2013a,b, 2015). The fear is that particles may not be captured by the magnetic separation process and that it could therefore lead to undesirable side effects (whether in the short or longer term), or that the pathogen could need to be identified before the procedure was used.

This presentation introduces a strategy for the evaluation of any potential risks associated with the procedure, and the results of a comprehensive study on risk assessment (Herrmann et al., 2016). We investigate selected *in-vitro*, *ex-vivo* and *in-vivo* models and their advantages and disadvantages and show some procedural changes and medium optimisations (Fig. 1) that help to overcome most of the risks and deliver a valuable balance between risk and benefit.

Subsequently, we present a new approach to the use of the theranostic potential of the magnetic blood purification procedure. This will also include an examination of the composition of a material used for magnetic separation that can help to quickly separate and isolate many pathogenic bacteria without any need for identification of the pathogenic agent in advance.

Finally, we introduce a plan for the transfer of this approach into hospitals, and discuss the feasibility of *in-vitro* and *ex-vivo* models in detail.

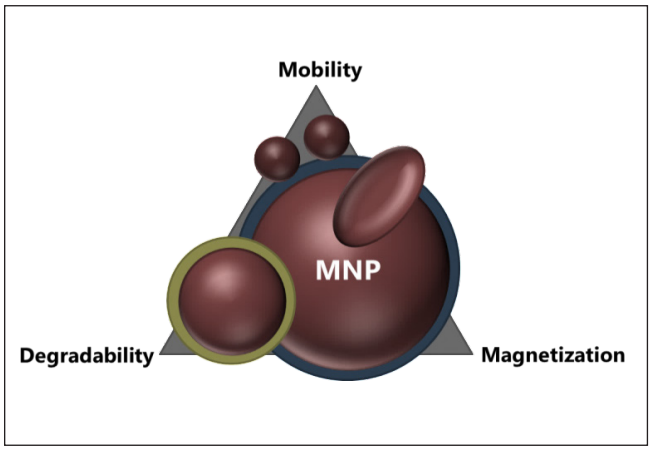

Fig. 1: Optimisation of magnetic agents on the basis of mobility, magnetisation and degradability

References

Herrmann, I. K., Urner, M., Graf, S. et al. (2013a). Endotoxin Removal by Magnetic Separation-Based Blood Purification. *Advanced Healthcare Materials 2*, 829-8252.

Herrmann, I. K., Schlegel, A., Graf, R. et al. (2013b). Nanomagnet-based removal of lead and digoxin from living rats, *Nanoscale 5*, 8718-8723.

Herrmann, I. K. Schlegel, A., Graf, R. et al. (2015). Magnetic separation-based blood purification: A promising new approach for the removal of disease-causing compounds. *Journal of Nanobiotechnology, 13*, 49.

Herrmann, I. K., Beck-Schimmer, B., Schumacher, C. M. et al. (2016). In vivo risk evaluation of carbon-coated iron carbide nanoparticles based on short- and long-term exposure scenarios. *Nanomedicine 11*, 783-96.

Putting the 3Rs into practice – is cash the problem?

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Working with animals from the simplest single-cell organisms all the way mammals is, in a variety of ways, part of the contemporary process of scientific discovery. It is (and ever was) the subject of a broad variety of criticism. If we are to understand the application of the 3Rs in research, we need to discuss the various experimental approaches: Research with animals involves either 1) research into the basics of physiology, anatomy or behaviour; in this case, the attention is on the acquisition of knowledge – but not necessarily on any application of that knowledge (basic research) or 2) research into physiology, anatomy or behaviour in relation to a particular problem, with a specific aspect of application – frequently a medical problem – and potential approaches to solutions, such as diagnostics and treatment (applied research). Research carried out with animals is aimed at a particular discovery a) for the species of animal upon which the research was undertaken, b) for translation to other animal species, and potentially as a representative for large groups of animals (e.g. mammals, vertebrates, etc.) and c) for translation in the anthropocentric sense (as the word is mostly used) – i.e. transmissible to humans. In cases b) and c) the transmissibility of the results to the (other) target species (including humans) must have a good scientific basis. Furthermore, we must always differentiate between stressful and non-stressful animal experiments.

Regardless of the purpose of the research, laboratory animals must be subjected to as little suffering as possible, and their inherent dignity must be respected. Here, it is a matter of carrying out the research on the animal in as humane a way as possible. The 3R principle has established itself as the "Guiding Principle" for the reduction of suffering in this case. The 3R principle is based on a concept put forward by the researchers W. M. S. Russell and R. L. Burch in 1959, in their book on "The Principles of Humane Experimental Technique". Specifically, the Rs stand for "Replace", "Reduce" and "Refine".

Replace refers to the replacement of animal experiments by alternative methods (which may also include experiments on other animal species, who will either suffer less or not at all), *Reduce* is aimed at minimising the number of animals required, while *Refine* refers to the lessening of stress and an improvement in the living conditions of the animal. The 3R principles have been recognised for a long time in laboratory animal science already and have gained an increasing foothold in national and international law over the past few years.

When it comes to the reasons for animal experiments, it seems that that we have overarching approaches to the implementation of 3R and options that are specific to the different research directions. In research into the general processes of life, we should consciously restrict our activities as much as possible to animals who have no ability to suffer (or very little ability to suffer). However, this alternative option is not available in research that aims to understand a particular species and its physiology, anatomy or behaviour. In translational research, the animal species with the lowest ability to suffer must always be chosen from those animal species models that are suitable. If the research is intended for application to human beings, the choice is often limited. *Replacement* is apparently only considered if the alternative methods can answer the questions that apply to this species in particular. In *Replacement*, the initial experimental steps are often undertaken "*in vitro*" or "*in silico*"; it is often only essential for the discoveries to be verified in a living animal organism after the findings have been well secured.

Research in veterinary medicine is undertaken on an animal for the benefit of that animal. In this case, *Replacement* is subject to relatively narrow limits, since many diseases are species-specific, and may even only appear in certain breeds within a species. The majority of these considerations therefore relate to experiments that apply to translational research aimed at the acquisition of medical knowledge for human beings. Even though this type of research has been carried out for several hundred years, it has always had to deal with the question of which findings are applicable to humans. A strong legal basis has been established for translational animal experiments since the Nuremburg trials, and the principles for experiments on humans that were established for the first time in 1947 in connection with these. The Code formulated at that time was a result of proceedings against doctors, who were standing before a US military tribunal because of crimes against humanity during the Nazi era. On this basis, the World Medical Association adopted a guideline for biomedical research on human beings in 1964. This is known as the "Helsinki Declaration" and is binding worldwide. It specifies that all the experiments (including animal experiments) necessary in order to exclude any danger to humans must have been carried out before any experiments are undertaken on human beings. In the years that followed, animal testing therefore grew to be a standard procedure in translational research. While there are many examples available of the successful acquisition of findings through animal experiments, opponents of animal testing have also repeatedly cited individual examples to the contrary. The strict ethical and legal limits set on experiments undertaken on human beings makes it clear why animal experiments are justifiably so important in translational research!

The 3Rs now play an important role in this challenging context, as its goal is to facilitate (translational) research while simultaneously minimising the suffering of the animals that are still being used. If we look at the 3R Principles within the param-

eters of this research area, we can find examples of *Replacement* in the use of computer models and *in vitro* techniques, e.g. using cell cultures or organ-like tissue cultures derived from humans. *Reduction* can be achieved by modern imaging techniques, which allow processes to be observed repeatedly in the same animal, and *Refinement* includes the improvement in scientific research methods, animal breeding and husbandry methods and the care and treatment of laboratory animals. Altogether, application of the 3Rs can avoid suffering, pain, fear, stress and injury in translational research. Another positive effect is that the validity of the animal experiments is also improved if tests are undertaken on fewer suffering animals. An additional important aspect of Reduce would involve the parallel use of laboratory animals to check various hypotheses; the Institute of Laboratory Animal Science (LTK) has taken on a leading role in this respect and initiated the introduction of the *Animatch* System at the University of Zurich (with an option to expand the system to Swiss institutions). This system facilitates the sharing of any parts of organs or animals that have not been used within the framework of an experiment.

So how can we now promote the 3Rs beyond what has already been achieved? Do we need more money to support the implementation of the 3Rs in Switzerland? If we consider this directly, the funding provided by the Federation for the improvement of animal welfare within the framework of the 3Rs (i.e. the grants provided for the current 3R Research Foundation) is within the limits of a small SNSF research grant, at best. Initially, therefore, it seems that there is a disparity here. Simply the provision of finance at a level equivalent to an SNSF research support grant for one single 3R research project in each of the 3Rs per year would represent a significantly greater sum than the total financing that has been available up to now for the entire 3R Research Foundation. Nevertheless, we can assume that improvements in animal welfare were frequently achieved within the 3R framework in the research projects promoted by the SNSF and others. Because of the lack of transparency, clear regulations would be helpful in relation to project applications. Implementation of the 3Rs could be a general component of an SNSF research application and research report in Biomedizin. Clear evidence must be provided for how the planned experiments would follow the principles of 3R. This could result in a preferred promotion. Furthermore, projects could be awarded with a bonus if different groups could exploit the same animal experiment (with the suffering remaining at the same level). This would significantly promote cooperation with regards to animal experiments. A model could conceivably be designed in which the research grant and the animal experiment application were linked. This would also have the advantage of preventing those situations where the funding has been promised but the experiment cannot be carried out because of a lack of approval, e.g. if there is a negative balance of interests.

Let us now turn to 3R research areas in order to gain an overview of projects that are worthy of support, and therefore gain some indication of the financial requirement. In the *Replacement* of translational experiments, these would certainly involve computer models, new Omics methods, improved cell cultures, better human diagnostics and also, in special cases, Phase 0 Studies. Few of these methods are regarded as direct 3R methods and are therefore undertaken via other forms of financing – if they receive any support at all. It would certainly be helpful if studies could be undertaken on the industrial, university and SNSF levels in this respect, to learn how many animals could be spared by these modern developments by now. A model for the future would incorporate the question of 3R in the biomedical research applications, as discussed above.

In the area of a *Reduction* in the use of laboratory animals, some attractive opportunities have arisen from the world of *in vivo* imaging: fluorescence, luminescence, MRI and CT now help in many experimental systems to observe animals over the course of time, without always having to kill certain cohorts. Unfortunately, it is very expensive to acquire, maintain and operate this equipment. Even though the significance of modern imaging techniques for animal welfare is clear, this option is still not adequately supported by funding, e.g. by offering a clearly-defined bonus for research application that use such techniques, or by providing enough financial support for proven qualified core units with trained personnel. Even the research studies that now use imaging to correlate the course of diseases with the human data (and earlier animal experimental) also urgently require funding. A reduction in the number of animals can be achieved directly by breeding transgenic animals, as well as through optimised breeding procedures and cryo-conservation. There is a lack of support for the development of the relevant software, and cryo-conservation is only supported financially at a few institutions. Reduction can also be achieved by optimising the design of experiments – most successfully through better training and clearly-directed support. The education and training provision must include far stronger teaching of the necessary statistical methods and experimental designs. The LTK has taken on a pioneering role here too – it provides information on 3R methods via its website, via the Swiss3R-Network and via Twitter. Collaboration between the Swiss-3RNetwork and the online ScienceMatters Journal facilitated the publication of 20 observations relevant to 3R. Even now, bachelor-level courses in biology/biomedicine already include an introduction to the 3Rs, including practical discussions on animal ethics. Experimental scientists are provided with opportunities for reduction on new special courses based on experimental design/power calculation and the planning of breeding programmes.

Nevertheless, when it comes to the direct financial support provided for 3R, most success can be achieved in the short term for animal welfare in the area of *Refinement*. A large number of experiments involving extremely different methods are carried out within the framework of basic and translational research, and we need to strive to optimise the structure of each experiment in relation to animal welfare. The range of this task must not be underestimated. What form of anaesthesia and analgesia can researchers use within the framework of a particular experiment

without it affecting their experimental results? Even this apparently simple question is often difficult to answer.

Finally, we should not forget the role of the skilled Animal Welfare Officers at the research institutions. Their control function is not only far too undervalued, they also help researchers to find the best solution in regard to animal welfare before they even embark upon their experiments. Nevertheless, the variety of potential experimental scenarios mean that their work is very challenging, and they need enough time if the job is to be more than just ticking the box against standard points. The problem applies to members of the animal experiment committees as well. These committees must be of a size and constitution that enables them to carry out a review process in line with the 3R Principles.

In conclusion, we can assume that there will be a considerable financial burden involved with the implementation of the 3Rs at Swiss research institutions. The exact amount of financial outlay is difficult to quantify, since a large amount of money for 3R is already hidden in research applications and in personnel posts at research institutions. Nevertheless, it seems as if further improvements could be made on the level of both the federal research funding as well as the research and educational institution. Resources are required in order to improve non-invasive research on humans, thus avoiding the need for the corresponding translational animal experiments. Research funding is required in order that any animal experiment that may still be unavoidable can be optimised in terms of the 3Rs. Resources are required to promote the application of the 3Rs at the research institutions and for the education and training of new researchers and the ongoing training of those researchers who are already active.

In summary, we can say that a great deal has already been achieved for animal welfare over the past few decades, since the publication of the 3R concept by W. M. S. Russell and R. L. Burch. This has happened as a result of the consistent implementation of the 3Rs at research institutions. But it is precisely because the obvious, mostly "easy" problems have been solved by now that we still need funding; we can then improve the welfare of animals used for research by targeting the complex problems that remain unsolved, and thus do proper justice to the 3R concept.

Refinement – the forgotten R?

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The current position of the third R

The research that uses animals in experiments is primarily aimed at human beings; this is, for example, reflected in the balancing of interests for animal experiment application No 63, where the advantages for humans are weighed against the disadvantage of the animal. The advantages for humans arise from the long-term and overarching aim of the experiment as well as from the short-term specific aim, as set out in Clause 44.1 and Clause 44.2 at the outset of an animal experiment application.

The aims for human beings are set against the goals for the animals – i.e. animal welfare – which are defined in the animal experiment by the 3Rs. Within the 3Rs, the main focus for the last R (which involves the refining and improvement of experiments in order to reduce the stress for laboratory animals) is on the animal and the effect on its wellbeing. Refinement is, by definition aimed at the animal, its life, its normal routine, its welfare and its health while it is being kept and during the experiment.

Improvements and refinements in experiments, such as a further development of the methods being used, often occur in line with the progress of the research. However, these are very difficult to measure or to be appreciated from the outside, as these types of developments are often subsumed in the experimental aims listed for humans, and their significance for the animals is apparently often not consciously perceived by the researchers themselves.

On the other hand, researchers do make modifications to experiments and husbandry procedures to improve the conditions for the animals. These changes must not corrupt the biomedical or basic scientific experiment (approach), so modifications for the benefit of the animals are still subordinate to the approved, underlying experimental aim. It is very difficult to realise a serious simultaneous treatment of a scientific question aimed at animal protection within the framework of a biomedical or basic scientific experimental project and it is therefore pushed into the background. In other words, the researchers must concentrate in the first instance on the research aim and the hypotheses of their research, as already envisaged and approved. One result is that no effort is put into animal welfare improvements and they are not publicised to an equivalent extent in these experiments; they therefore fail to gain any external impact or relevance.

In addition, we also need to bear in mind that refinement measures driven by good intentions may prove to be counter-productive, because knowledge about the needs and behaviour of the animals is either completely absent, or insufficient, or it is not understood. This may be the case in particular with animal species such as the mouse, who do not play an important role for human

beings, except for research purposes, and who also live under unusual conditions and experimental influences in the laboratory.

The observation made by Nobel Prize winner Peter Medawar is particularly applicable here:

> *"The welfare of animals must depend on an understanding of animals, and one does not come by this understanding intuitively. It must be learned." (Medawar 1972)*

Interim conclusion

Efforts at Refinement largely remain untargeted, vague and scientifically vulnerable when they are integrated as a secondary concern, or as an appendage to research goals that are focussed on the benefits for humans.

Over 600,000 animals are still used every year in animal experiments in Switzerland alone, and we therefore require research proposals with a clear definition of refinement as an experimental aim, with the focus on "the animal under experimental conditions".

Scientific work leading to internationally recognised publication and an ability to exert a global effect on animals in experiments requires a platform that recognises refinement as an experimental objective and one that can ultimately also ensure that financing is provided.

This does not describe the current situation in Switzerland to any significant extent.

The current perception of the lack of importance of the third R and the situation with regard to financing for Refinement is described below.

Pain management in mice

Mice are the laboratory animals used most frequently (over 60%) worldwide. In Switzerland, over 100,000 mice are used every year in experiments with a severity level of 2 or 3, and we can assume that the great majority need to be treated with analgesics.

Our research group has, within the framework of several studies, developed a retarded-release preparation using the University of Zurich and University Hospital of Zurich's own resources in order to optimise the treatment of pain in mice. The outstanding efficiency and animal welfare benefits compared with the conventional methods were published in 2015 and have led to two awards for 3R and animal welfare, as well as great acclaim.

We wanted to make this analgesic available to all researchers in Switzerland for use on their laboratory mice, without having any interest in profit or other commercial aspirations. The preparation required further development and its tolerability needed to be ensured. We sought third-party funding to support these studies, as well as other procedures to optimise pain management.

Even though the application's plans for the refinement of pain management gained wide acceptance, and we received many feedback messages saying that the aims would be worthy of funding, we have failed to obtain any significant support for several years, mainly because of economy measures and reticence before the start-up of the 3R Competence Centre.

For example, the 3R Research Foundation in Switzerland has not funded any new projects since 2016 and is only supporting the projects currently in progress until they are concluded. This Foundation will be dissolved when the 3R Competence Centre opens.

In January 2017, the Swiss Federal Food Safety and Veterinary Office (FSVO) announced "that no independent research project applications would be accepted in 2017. However, the FSVO will invite applications for funding for research that is aligned with its own specialist strategies. This change from previous practice is taking place partly because of the intention of the FSVO to direct its departmental research more purposefully towards the specialist strategies, and as a result of savings measures in the federal administration system: if we funded additional statutory orders in combination with austerity packages, this would lead to financial shortages that would need to be compensated by savings in existing assignments". (See also the website of the FSVO[1])

Animalfree Research demands explicitly that no animal experiments should be undertaken, i.e. projects are excluded if they show no anticipated effects (or minimal anticipated effects) on replacement and/or reduction of animal experiments.

Interpharma, the association of Swiss pharmaceutical research companies does not provide any funding contributions for individual projects, but it does support the 3R Competence Centre – however, that is still in development, and the association therefore does not provide any funding at all at present. Nevertheless, Interpharma has shown an interest in the project, but no support has been forthcoming because of current economy measures and the generally difficult budgetary environment.

At present, therefore, studies on the optimisation of pain management in mice are only being pursued by means of the University of Zurich and the University Hospital of Zurich's own limited resources, under the conditions that are correspondingly restricted.

Estimation of stress in laboratory animals

The Swiss directive used to estimate the level of severity has been in place for over 20 years (Animal welfare information 1.04, Classification of animal experiments according to level of severity before the experiment begins (stress categories)). No update has appeared in respect of more recent discoveries or the general development. Levels of severity have also been prescribed in the EU, in Directive 2010/63/EU.

In many animal experiments, particularly those that are stressful, any classification of stress into severity levels is frequently difficult and often more emotionally based than scientifically confirmed. The quotation from Nobel Laureate Peter Medawar (see above) applies here too, if the word "learned" is replaced by "researched".

Our research into the estimation of stress in mice introduced us to a consortium of twelve research groups formed in Germany to scientifically study the stress involved in various widely-used animal experiment models and to work out principles for clas-

[1] https://www.blv.admin.ch/blv/en/home.html

sification. As well as defining measures to reduce the stress, the intention is also to establish options and instructions for the grading of the stress, from which classification in other comparable models could also be derived.

A joint application for this major project was made to the German Research Foundation (the DFG, which is the national institution equivalent to the SNSF in Germany); following an audit with an external review, this was approved. We are integrated into this project as the only foreign research group; our sub-project has been certified for excellence by the DFG, and the financing for our work has been agreed. This should have been taken on by the SNSF on the basis of agreements with the DFG, but we were advised by the DFG that the project would not be funded by the SNSF because certain formal requirements in the application were unfulfilled.

Conclusions

If Refinement measures are adopted within biomedical or basic scientific experiments, any perception, demonstrability and dissemination of the improvements are often lost in the overarching aims and advantages for human beings associated with the experiment, or they are not mentioned in the relevant publications, and are therefore of no use to the research community.

Funding is needed to provide refinement that focuses on the animal, and on its life and wellbeing during the experiment. Refinement must be defined as an experimental goal and recognised as a research aim.

No significant financial support is currently being provided for research into Refinement in Switzerland.

The resources provided in previous years by foundations and institutions for animal welfare and 3R have been stopped for economy reasons.

Resources have also been frozen in view of the planned 3R Competence Centre. However, we do not expect any invitations for proposals from this direction in the near future.

Research aims in which the defined aim is "Refinement" are not eligible for applications to the SNSF, as they cannot fulfil the requirements, aspirations and conditions of the SNSF *per se*. The task of the SNSF is to support basic research in Switzerland, with an aspiration of scientific quality. Its focus is on the benefits for human beings, and the use of animals is also included in many research proposals. Under these conditions, Refinement can only take place within the experiments that serve the aims of the basic research. From our experience of the current use of animals for the purpose of research to benefit human beings, we conclude that targeted scientific research that focuses on the animal and its wellbeing is required

It is very important for the actual welfare of laboratory animals that the third R is recognised as of key significance and that the necessary research is independently funded.

The new 3R Competence Centre

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How can the 3R Principles be integrated into the research of today and tomorrow? This is the question that the working group at swissuniversities set itself when SERI[1] and FSVO[2] tasked it with preparing a proposal for a national 3R Competence Centre. In fact, this question concerns the modalities for the integration of the 3Rs into the normal routine of the universities and their stakeholders: researchers, students and society. There is therefore a requirement, for a competence centre that is directed towards these three target groups, who could potentially work together towards the better implementation of the 3R Principles. The 3RCC we envisage should thus fulfil three important functions: research into and application of 3R solutions, the education and training of students and researchers, and honest communication on the development of the 3Rs. These three pillars will represent the main activities of the centre, and all three will deal with the 3Rs. In the area of research, the centre would wish to support and promote 3R projects up to the marketing stage; in the area of education, the centre would not only wish to facilitate the integration of the 3Rs into the training of researchers, but also into the education of students; finally, communications from the centre will be addressed openly to society in general, to the scientific community and towards politics and the media.

And how will a national *Competence Centre* be organised? Several universities are already active in the development of 3R Principles, but their activities are far too frequently limited to laboratories or auditoriums and are not coordinated on a national level. This restricts the real development of the 3Rs and their effects. The work of a national Competence Centre lies in promoting an exchange of information, coordination and the best practices of all the institutions. In order for this aim to become a reality, the whole project must be united under one roof. We have therefore envisaged a *Group of Partners* who are interested in the 3Rs and are active in this field: eleven universities, SAP[3], the Confederation, in the form of the Swiss Federal Food Safety and

1 Switzerland's State Secretariat for Education, Research and Innovation
2 Swiss Federal Food Safety and Veterinary Office
3 Swiss Animal Protection (SAP) Office

Tab.1: Overview of the 3RCC

Structure	
Members	Key institutions (universities: EPFL, ETHZ, FHNW, ZHAW, Universities of Basel, Bern, Fribourg, Geneva, Lausanne, Zurich and USI), SAP, FSVO, Interpharma.
Strategic Board	Establishes the strategy based on the inputs of the Executive Board and the Stakeholders' Advisory Board. It appoints the members of the Scientific Board, the Stakeholders' Advisory Board and the Executive Board. It makes decisions about the budget, spending and the financing of open and targeted calls for proposals.
Executive Board	Implements the strategy. It is composed of: coordinators from the universities, representatives of the SAFN (Swiss Animal Facilities Network), Network of Animal Welfare Officers, Institute for Laboratory Animal Science, "*Réseau des animaleries lémaniques*". It considers the expert opinions of the Scientific Advisory Board.
Stakeholders' Advisory Board	Composed of representatives from institutions that have a close connection with animal experiments but who are not themselves members of the group (e.g. the Swiss National Science Foundation, the Academies of Sciences, the Ethics Committee for Animal Experimentation, animal protection associations). It advises the Strategic Board.
Scientific Advisory Board	Composed of 5 to 7 internationally recognised 3R experts, at least two of whom have experience as SNSF or CTI experts. It uses its scientific expertise to support the Executive Board. It is responsible for the evaluation of the proposals received within the framework of a call for bids.
Directorate	The directorate is located at the University of Bern and is composed of: a director, a communications expert, a scientific employee and a technical employee. Their main functions include: representation, communication, administration of calls for proposals, implementation of the operational decisions, coordination, gap analyses, dissemination, budget planning.
Financing	The Confederation, via Art. 15 FIFG, FSVO, SAP, Interpharma, universities.
Activities	
Research	The 3RCC promotes research by issuing calls for proposals for research projects. Every year, it organises one open and one targeted call for proposals, which will be awarded in the ratio of 2/3 : 1/3 of the research budget respectively.
Education	The 3RCC creates synergies between Swiss institutions and develops new skills, in close collaboration with existing operators. In particular, it: catalogues what is already on offer, identifies gaps in 3R education, coordinates the creation of innovative courses and supports research into 3R education.
Communication	The 3RCC will: develop tools (e.g. performance indicators) to evaluate progress in 3R education and research; organise information events; maintain regular contact with external stakeholders; award a prize every year for the best performance in the application of the 3Rs.

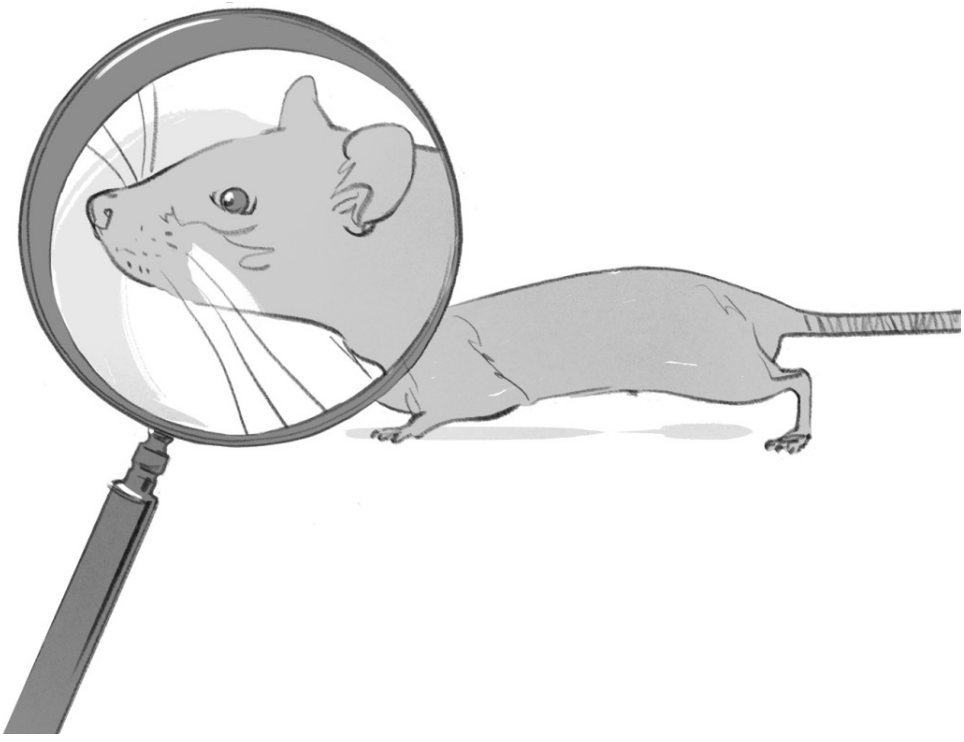
Veterinary Office FSVO and the Industry, through Interpharma. These partners and members of the group contribute financial or other resources to bring the centre into being. For operational purposes, the general assembly elects a *Strategic Board* and an *Executive Board*. As the superordinate body, the Strategic Board will bring together the representatives of each member institution and set the strategy for the centre. The function of the Executive Board will be to represent the voice of the centre at the universities; it will be made up of the coordinators from the universities who are active in the 3R sector. A Scientific Advisory Board and a Stakeholders' Advisory Board, who are not members of the group, complete the organisation of the centre; the *Scientific Advisory Board* will be responsible in particular for evaluating the received applications for funding, while the *Stakeholders' Advisory Board* will issue a statement on the strategy of the centre and its development towards increasing the impact of the 3Rs. Coordination and administration will be undertaken by a directorate located at the University of Bern.

The 3RCC and its organs are designed so that the research, education and communication activities can all be undertaken efficiently. *Research* will be supported by financing projects, either as submitted by researchers or in reply to an invitation from the centre for proposals. Projects that are small but of a high quality are just as eligible for support as projects aimed at the dissemination of technologies that are already established. The strategy in the area of *Education* will be implemented by the Executive Board and by the coordinators, who will look after the flow of information to and from the universities; the directorate is responsible for the national coordination. Strategy in the area of *Communication* will mainly be implemented by the directorate, in collaboration with the communications offices at the universities.

By establishing the 3RCC, Swiss universities, the Confederation and the other partners confirm their commitment to be more respectful of the dignity of the animals used in animal experiments. The 3RCC will be able to close existing loopholes – and to do that at the national level: animals will be better protected, research will be stronger and education will be more efficient; it will be easier to develop innovative technologies, and society will be better informed.

Presentations of the 11th Conference on Animal Testing

The 3R Competence Centre (3RCC) – Better Research with Fewer Animal Experiments?



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Introduction

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The launch of the new 3R competence centre (3RCC) means that after more than 20 years, the 3R principles are finally to be enforced as set out by legislators under Art.22 of the Animal Protection Act back in 1993: The Confederation shall work with universities and industry to promote and support the development, recognition and application of methods that replace animal experiments, that require fewer test animals, or that cause less distress to the animals involved.

Replacement methods have hardly been used in Switzerland up to now despite the fact that they have proven economic and scientific potential and are cheaper and faster. That is set to change. On a scientific and economic level, replacement methods offer much greater possibilities and uses than animal experimentation. It is no coincidence that the EU and U.S. are investing considerable sums in developing and implementing such methods.

For animal lovers and animal welfare campaigners the key question is whether the new competence centre will lead to a significant decline in animal experiments. And secondly whether it will successfully link up industry and higher education institutions in such a way that the 3Rs, in particular the replacement methods, become a priority in research activities. Academic research in particular has seen increasing numbers of animal experiments in recent years and should now take a more active role in this area. We will consider these and other aspects with knowledgeable speakers from Switzerland and abroad at our conference.

What the FSVO expects of the 3RCC (3R Competence Centre)

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The 3R principles[1] (replace, reduce, refine) should be implemented in every animal experiment. In Switzerland, researchers are obliged to minimise the number of animals used for animal testing. Alternative methods to replace animal testing should be used where available. Where animal experiments are absolutely necessary, the suffering of animal subjects should be kept to an absolute minimum.

All stakeholders, researchers, research funding agencies, the pharma industry and public authorities are working together to replace animal experiments, to reduce the number of animal experiments performed and to develop animal experiments that cause less pain and suffering. To support the implementation of the 3R principles, the Federal Council recommended the creation of a national 3RCC[2] competence centre.

Following a number of workshops, the Conference of Rectors swissuniversities was mandated by the federal government (State Secretariat for Education, Research and Innovation SERI and the FSVO) to develop a structural concept for a new national 3RCC. The new 3RCC was set up in March 2018. It is designed as a network of 11 higher education institutions and is supported by the institutions themselves, the pharma industry (Interpharma), the federal government and Swiss Animal Protection SAP.

The FSVO has high expectations of the new 3RCC, in particular with regard to the key elements of education, communication and 3R research. The core element in ensuring effective and sustainable improvements to the welfare of laboratory animals and in reducing the number of test animals is sound *education and training* of researchers. The 3RCC's close links to universities will allow the topic of 3Rs to be included in the curriculum for students on all science and medicine courses from an early stage. The aim is to establish a 3R culture in Swiss laboratory animal facilities, research institutes and laboratories.

To achieve this, the 3RCC needs to develop and implement a 3R training strategy, which should take into account the different education and training formats and ensure coordination between existing university 3Rs teaching programmes.

[1] 3R principles – Replace, Reduce, Refine

[2] Future of the 3R research foundation and alternative animal testing methods, Federal Council report in response to the postulate 12.3660 of the National Science, Education and Culture Committee of 17.08.2012

This key role in education, training and development will allow the 3RCC to become a centre of expertise on the animal-friendly handling of laboratory animals and more broadly to establish itself in the field of 3Rs as a knowledge and experience sharing platform for the animal testing community. The 3RCC needs to develop a communication concept which should include the establishment of a professional contact point for the various stakeholders and which in future will allow structured *communication* with stakeholders (students, researchers, the public, the media, public authorities and policymakers).

This active internal and external communication will ensure maximum transparency both within the research community and among the general public. Finally, we expect the 3RCC to develop international links with other 3R competence centres in Europe and worldwide to share knowledge, experience and methods.

A *3R research* strategy should be developed to identify and initiate high-quality competitive research projects that take into account all areas of the 3Rs (replace, reduce, refine). Particular importance should be attached to projects that develop new approaches or technologies right through to implementation and which are not supported by other funding instruments (such as those of the Swiss National Science Foundation). The focus should clearly be on researching alternative methods. In the regulatory field, the 3RCC should act as a catalyst for the implementation of non-animal methods. As long as animal experiments are unavoidable, the 3RCC should support studies and projects that develop animal-friendly methods that aim to significantly and sustainably reduce the suffering of laboratory animals. The 3RCC should also promote methods that aim to optimise the number of animals used in order to obtain meaningful research findings.

The 3RCC should develop suitable evaluation instruments and key indicators for the field of 3Rs to measure and monitor the progress made in teaching and research. In addition, a set of basic principles should be established defining how "unpublishable" results in all 3R research fields should be managed.

The FSVO looks forward to working closely with the 3RCC to see progress in the implementation of the 3R principles and to support the activities of all stakeholders.

Swiss 3R Competence Centre: Advancing research & education on 3Rs

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The Swiss legislation on the protection of animals requires that all person taking care of animals, takes as much as possible into account their needs and ensure their wellbeing as far as the scope of their use allows it. Considerable progress took place in the last decade at an international level for the refinement, reduction and replacement (principles of 3Rs) of animal experimentation for regulatory purposes. On the 27 March 2018, the Swiss 3R Competence Centre (3RCC) has been founded to further promote the principles of 3R in the areas of research and education.

Under the presidency of Dr. Kathy Riklin, member of the Swiss National Council, the 3RCC represents an association of academia, industry, regulators, government and animal welfare association including the eleven most important Universities and Higher Education Institutions from Switzerland, the Swiss association of pharmaceutical industry (Interpharma), the Swiss Federal Food Safety and Veterinary Office (FSVO) and, the Swiss Animal Protection. The 3RCC also benefits from an important support from the Swiss State Secretariat for Education, Research and Innovation (SERI), as it represents a scientific centre of national importance working on a non-commercial basis according to article 15 of the Federal Act on the Promotion of Research and Innovation (RIPA).

Having its offices kindly hosted by the University of Bern, the Swiss 3R Competence Centre will subsidize scientific projects of quality and establish an educational program and communication strategy to promote the principles of 3Rs. A first call for scientific projects is planned for late 2018. In addition, through its educational program and communication strategy, the centre aims at making accessible to all those involved and/or interested on animal experimentation, up-to-date information on alternative methods to animal experimentation. Finally, the 3R Competence Centre will monitor progress made regarding the implementation of the principles of 3Rs in Switzerland and will offer its services to authorities, teaching bodies and other interested parties willing to gain additional information on the principles of 3Rs and on alternative methods to animal experimentation.

The Berlin-Brandenburg Research Platform BB3R – research and graduate training since 2014

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The Berlin-Brandenburg research platform BB3R, which was set up at the FU Berlin in 2014, pools 3R expertise in the Berlin-Brandenburg region and promotes systematic research in this field. The integrated graduate school is the world's first to offer a structured qualification in the 3Rs for young scientists, PhD students and junior professors. The platform received support from Germany's Federal Ministry of Education and Research in the form of seed capital.

Eleven founding members conduct research in the fields of skin disease models, immunology, human-on-a-chip, nanotoxicology, *in silico* analysis of active compounds and drug design (reduction/replacement). For animal experiments that are not replaceable, the platform develops refinement measures and studies the impact of multiple experiments, which also aim to reduce the numbers of laboratory animals. Besides this group of experienced scientists, the platform has also comprised PhD students and three junior professors from the outset. The consortium is boosted by nine renowned scientists who are associate members. The first scientists to complete the graduate programme already hold senior positions or have been offered professorships and can therefore pursue their 3R-research in Germany and internationally.

In addition to the research project, the graduate programme also includes regular PhD symposia, 3R seminar series and annual spring schools. Particular emphasis is placed on teaching students about all aspects of the 3Rs. The programme is organised under the aegis of the FU Berlin's Dahlem Research School (DRS), which offers courses on general skills (e.g. presentation, statistical analysis, good scientitfic practice). External PhD students are also accepted at the graduate school provided they work in one of the 3R fields and meet the DRS quality requirements.

Below are some examples of the consortium's research activities.

The Schönfelder project team is working on the development of individualised pain management for mouse strains as mice are currently the most widely used laboratory animals. Based on the results of this study, it should be possible to give more precise dosage recommendations for Buprenorphine for different mouse strains in order to keep the pain experienced by mice during experiments to an absolute minimum (refinement).

The Schäfer-Korting project team is building tumour models of non-melanoma skin cancer and head and neck tumours to study the absorption and effect of cytostatic drugs. In these cases, the translatability of results from animal experiments is by far the lowest. This is to be counteracted with an integrated test strategy, where in the preclinical phase a drug candidate is initially tested for tolerability and suitability on a 3D model. Only the substances that are successful in these tests would then have to be tested for tolerability on animals in order to exclude adverse effects as far as possible when first used on humans.

Human lung cultures as an example of research at the new Charité 3R Centre

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Inflammation of the lungs (pneumonia) is one of the five most common causes of human death worldwide and is a widespread disease in Europe. Studies involving large patient cohorts show that the mortality rate of this disease has remained unchanged at around 13% for over sixty years. This is especially striking considering the significant progress made in basic research, the advancements in intensive care medicine and the availability of a number of pneumonia vaccines.

The bacterium *Streptococcus pneumoniae* remains by far the most common isolated pathogen. Zoonotic viral pneumonia pathogens in particular, such as influenza viruses and SARS and MERS coronaviruses, still show significant epidemic and pandemic potential.

If we want to study these pathogens, we should bear in mind that *Streptococcus pneumoniae* is a strictly human pathogen, while the viruses mentioned above exhibit strong species

specificity. Studying many of these pathogens using animal models therefore has limited relevance to humans; for some the available models are very insufficient (Zscheppang et al., 2018; Hocke et al., 2017). These examples show that animal models have significant limitations in their translatability to humans, at least in relation to certain diseases. In addition, there are major differences in key physiological and pathophysiological characteristics between species. The use of experimental subjects that are as homogenous as possible and various other aspects partly explain why many methods that are successful in basic and preclinical research fail when they are translated into clinical practice. Besides ethical questions, there are thus many scientific reasons to actively develop viable alternatives to animal testing. The new Charité 3R Centre therefore aims to consolidate the faculty's activities in all areas of the 3Rs with a focus on the systematic development of alternative methods. This involves seeking interdisciplinary cooperation with local and external partners from the outset. The stakeholders believe that development of the models themselves poses a major scientific challenge and that significant efforts need to be made to standardise and distribute them (e.g. cryopreservation).

As an example, a cultured *ex vivo* infected human lung tissue is being presented here. This allows us to study the relevant infection processes of bacteria (Nerlich et al., 2018; Peter et al., 2017; Fatykhova et al., 2015; Szymanski et al., 2012) and viruses (Berg et al., 2017; Knepper et al., 2013; Hocke et al., 2013a,b; Weinheimer et al., 2012) in pneumonia. It should be noted that in its current form this model obviously also has inherent limitations as it does not take account of breathing or circulation. But it does allow us to compare the replication of viral and bacterial pathogens using clinical isolates without the need for adaptations, which enables us to identify the true pathogen-specific virulence factors. Using spectral confocal microscopy imaging, we can identify the pathogen tropism and local alveolar damage. This high spatiotemporal resolution microscopy allows the movement and localisation of mitochondria and the development of apoptosis in three-dimensional tissue to be observed live in the presence of fluorescent pathogens. Traditional methods, for example to measure inflammatory response (e.g. interferons) are also used. We can analyse molecular mechanisms in differentiated tissue using e.g. GFP-tagged proteins which are transmitted via viral transduction. On the whole, this method not only constitutes an alternative to traditional animal testing, it also (subject to its own limitations) allows us to obtain information of great biomedical relevance, which animal models cannot provide.

References

Berg, J., Erkens, C., van Rest, J. et al. (2017). Tyk2 as a target for immune regulation in human viral/bacterial pneumonia. *Eur Respir J 50*, 1700977. doi:10.1183/13993003.00977-2017

Fatykhova, D., Rabes, A., Machnik, C. et al. (2015). Serotype 1 and 8 pneumococci evade sensing by inflammasomes in human lung tissue. *PLoS One 10*, e0137108. doi:10.1371/journal.pone.0137108

Hocke, A. C., Berg, J., Becher, A. et al. (2013a). Reply to Fujino et al. *J Infect Dis 207*, 693-695. doi:10.1093/infdis/jis740

Hocke, A. C., Becher, A., Knepper, J. et al. (2013b). Emerging human middle East respiratory syndrome coronavirus causes widespread infection and alveolar damage in human lungs. *Am J Respir Crit Care Med 188*, 882-886. doi:10.1164/rccm.201305-0954LE

Hocke, A. C., Suttorp, N. and Hippenstiel, S. (2017). Human lung ex vivo infection models. *Cell Tissue Res 367*, 511-524. doi:10.1007/s00441-016-2546-z

Knepper, J., Schierhorn, K. L., Becher, A. et al. (2013). The novel human influenza A(H7N9) virus is naturally adapted to efficient growth in human lung tissue. *MBio 4*, e00601-00613. doi:10.1128/mBio.00601-13

Nerlich, A., Mieth, M., Letsiou, E. et al. (2018). Pneumolysin induced mitochondrial dysfunction leads to release of mitochondrial DNA. *Sci Rep 8*, 182. doi:10.1038/s41598-017-18468-7

Peter, A., Fatykhova, D., Kershaw, O. et al. (2017). Localization and pneumococcal alteration of junction proteins in the human alveolar-capillary compartment. *Histochem Cell Biol 147*, 707-719. doi:10.1007/s00418-017-1551-y

Szymanski, K. V., Toennies, M. Becher, A. et al. (2012). Streptococcus pneumoniae-induced regulation of cyclooxygenase-2 in human lung tissue. *Eur Respir J 40*, 1458-1467. doi:10.1183/09031936.00186911

Weinheimer, V. K., Becher, A. Tönnies, M. et al. (2012). Influenza A viruses target type II pneumocytes in the human lung. *J Infect Dis 206*, 1685-1694. doi:10.1093/infdis/jis455

Zscheppang. K., Berg, J., Hedtrich, S. et al. (2018). Human pulmonary 3D models for translational research. *Biotechnol J 13*. doi:10.1002/biot.201700341

The Tox21 concept: Toxicology without animal experiments

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Because the development of new drugs using animal experiments is becoming increasingly expensive and because many such drugs have proven toxic or ineffective when used on humans, experts from the US Academy of Sciences tried to develop a new scientific concept for drug development free from the ethical problems of animal testing. The results were published in 2007 in "Toxicity Testing in the 21st Century – A Vision and a Strategy". In it, the experts concluded that in a not-so-distant future all routine toxicity testing would be conducted in human cells or cell lines *in vitro* by evaluating perturbations of cellular responses in a suite of toxicity pathway assays. In other words, animal experiments would be replaced by studies using human cells and tissue.

A key element of this Tox21 concept was the suggestion that the toxicity of foreign substances, drugs and endogenous substances, such as hormones, is based on adverse outcome pathways (AOPs).

As it has been possible to grow human cells and tissue under physiological conditions for 20 years, the AOP concept was extensively tested and confirmed by scientists at universities, in industry and in public agencies The EU AXLR8 project, which I coordinated at the FU Berlin from 2009-2012, also contributed to these efforts in Europe. An initial success based on the AOP concept was the development of new OECD testing methods for skin sensitisation which used human cells and tissue so that animal experiments were no longer required. The Tox21 concept led to a paradigm shift internationally, and AOPs now have to be taken into account when developing new toxicological test methods in all areas of pharmacology and toxicology, namely using human cells and tissue. Of course, this is also resulting in the replacement of animal experiments that were previously compulsory.

Multi-organ chips, on which several miniature human organs can be grown, have played a major part in this advancement. They are now used in the development of new drugs and to assess the risks of ingredients of cosmetics for which animal experiments are no longer permitted in Europe.

The US Academy of Sciences has since reviewed the Tox21 concept and in 2017 published the study "Using 21st Century Science To Improve Risk Related Science". In it, the experts conclude that the Tox21 concept has clearly improved the quality of risk assessment for the protection of human health and the environment. To put this insight into practice, the major US federal agencies – FDA, EPA and NIH – launched extensive support programmes for the development of new safer drugs and chemicals in consumer products at the end of 2017. It is hoped that Europe and other industrial nations will soon follow suit, as the Tox21 concept confirms the scientific superiority of animal-free methods.

In search of alternatives to foetal bovine serum – light at the end of the tunnel

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The use of serums as supplements to culture media has long been routine practice in cell and tissue culture. Serums, in particular foetal bovine serum (FBS), supply cultures with hormones, growth and attachment factors, binding and transport proteins, additional amino acids, vitamins and trace elements.

However, the use of foetal bovine serum also has a number of disadvantages. Serums may contain bacterial toxins (endotoxins) and undesirable microorganisms such as bacteria (including Mycoplasma), viruses and prions. Furthermore, there are enormous seasonal and geographical variations in the qualitative and quantitative composition of individual serum batches, which often necessitates costly and time-consuming batch tests. The serum thus introduces an undefined mixture of biologically active substances into a defined culture medium.

Foetal bovine serum is a by-product of the beef industry. As such, the serum market is dependent on many external factors. The question is therefore increasingly being asked as to whether the worldwide demand for foetal bovine serum in research and the biotech industry can be met at all. Scandals concerning adulterated foetal bovine serum that have recently come to light have further heightened concerns about the purity and quality of serums.

The most serious drawback, however, is the serum extraction method. Foetal bovine serum is harvested from the foetuses of pregnant cows. It is estimated that approx. 800,000 litres of foetal bovine serum are required every year, which equates to 2 million bovine foetuses. The ethical concerns around serum extraction have gained traction in recent years and a range of alternatives have been identified in order to reduce the use of, and/or fully replace, foetal bovine serum to lower the annual consumption figures for bovine foetuses in line with the 3Rs.

Despite many innovative approaches and the development of serum-free media for a wide range of cells, the use of foetal bovine serum still remains the method of choice in cell culture.

To avoid the disadvantages of using serum, to create defined and controlled culture conditions and for animal welfare reasons, the search for alternatives to the use of serum in cell culture has intensified in recent years. We now have a promising solution on the horizon.

Human platelet lysates (hPL), which are enriched with platelet-derived growth factors, are the latest development. Serum contains a wide range of mitogenic growth factors, which are released from activated platelets during the coagulation process. Many of these factors were identified early on as essential mitogens in serum-free culture media. Following on from this observation, human platelet lysates have established themselves as an adequate substitute for foetal bovine serum in a wide range of different culture systems in recent years. Human platelet lysates are derived from expired donor platelets collected in blood banks. Donor platelets have a shelf life of just five days within which they may be used clinically. This means that donor platelet concentrates are regularly available.

The use of human platelet lysates (hPL) in cell culture should be considered in the context of platelet physiology. Platelets produce a range of growth factors, which they store in their α-granules and release when they are activated. These factors play a key role in stopping bleeding and in the subsequent wound healing process.

It is well known that the coagulation process in the harvesting of raw serum is critically important to the quality of the serum. It can therefore be assumed that the factors detected in the serum that are essential to the proliferation of cultured cells, such as EGF, PDGF, FGF, TGF-β and VEGF are of platelet origin. The high level of specific growth factors therefore makes human platelet lysates an excellent, if not fully defined, substitute product for cell and tissue culture.

As mentioned above, hPL are derived from expired platelet concentrates obtained by apheresis. Plateletphereses are carried out in certified blood banks. This means that once the shelf life has expired, a quality-tested starting product manufactured in accordance with European guidelines and certified for therapeutic use (platelet donation) is available. The donor platelets are washed, resuspended in a saline solution and lysed in a simple freeze-thaw process. The lysates can be added as a serum replacement to basal media, such as MEM, DMEM, DMEM/Ham's F-12 and RPMI-1640 in a concentration of 5% (v/v).

In addition, human platelet lysates are a culture system based purely on human factors. Such systems are free from any animal-derived components and are thus particularly suited to stem cell culture and tissue engineering.

Foetal bovine serum cannot be extracted directly or manufactured. However, the potential availability of expired donor platelet concentrates from blood banks and the ease with which lysates can be produced give us reason to be optimistic that this innovative and successful serum replacement method will soon be used more widely in cell culture laboratories.

Recombinant antibodies (research, production and implementation)

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Replacing animal experiments in biomedical research is a long and complex process. For example, recombinant antibody technology has allowed antibodies to be produced without the use of animals for more than 20 years. Application of this technology could significantly reduce the number of animals used in laboratories, while facilitating research tasks. However, it has not yet extended to basic research laboratories in the biomedical field, chiefly because of its relative sophistication and cost. Our overall objective is to promote the replacement of animal-derived antibodies with recombinant antibodies produced entirely *in vitro*.

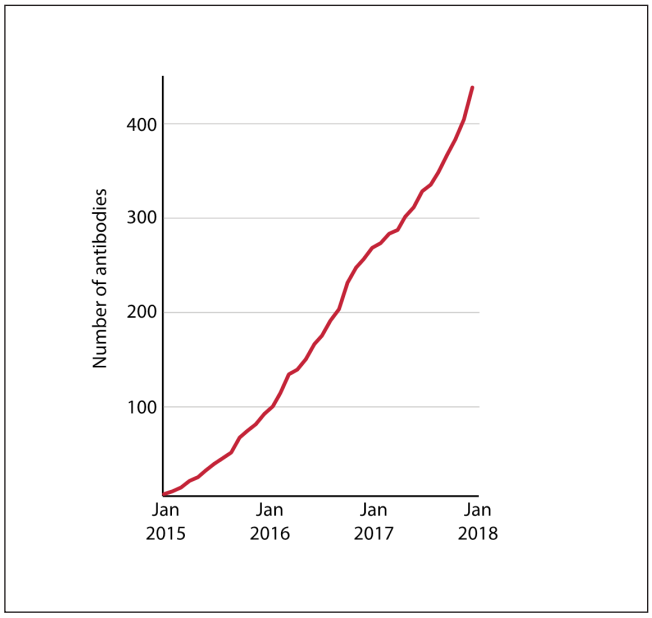

Fig. 1: Number of recombinant antibodies available at the Geneva Antibody Facility
https://www.unige.ch/antibodies

In 2014, we opened a university centre in Geneva to offer access to recombinant antibody technology to basic research laboratories, and to reduce the use of animals in research. The centre focuses on the discovery of new recombinant antibodies, of which it has produced hundreds (see Fig. 1).

A new project that is currently underway involves creating a complete database of all recombinant antibodies that have been discovered to date. This database will be linked to a production centre, which will provide access to all these antibodies. Our long-term vision is to create a completely open centre which will produce antibodies *in vitro* for the global scientific community. This example illustrates the potential and the difficulty of implementing a new technology to replace animal experiments.

The Scar in the Jar – an *in vitro* system to test antifibrotic substances

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Scars are recognisable because they look different from normal tissue. Scar tissue is the result of a rapid repair process and describes the accumulation of collagen fibres at the site of a wound healing reaction. This reaction did not evolve to look nice, nor to restore full function, but to rapidly restore tissue cohesion (Mother Nature's quick fix). Scar formation follows a relatively predictable process: following an injury, i.e. a break in the local tissue architecture (scratch, cut or crater), there is bleeding followed by haemostasis. Then comes the inflammatory phase, during which phagocytic cells seek out the lesion, clear out the bacteria and digest the damaged tissue; repair cells, fibroblasts and endothelial cells are then drawn to the wound (proliferative phase). At this point, the wound is a veritable construction site, with demolition and reconstruction work going on, and where in the end construction prevails. The building material is extra-cellular matrix, mainly collagen. Collagen forms fibre systems, which fill and bridge the wound defect, allowing cells to migrate and form a closed roof. The collagen fibres are more densely packed than normal tissue, which is why we can recognise scars with the naked eye as well as under a microscope. During the various phases of wound healing, the regeneration phase leads to wound closure. The collagenous scar then matures; it hardens but decreases in size. Surgical scars, for example, require at least a year before they turn from red to white. Every surgical intervention involves scarring in all affected layers of tissue.

Scars on the skin can significantly reduce the appearance and function of the skin's mobility and elasticity. However, if scarring encompasses a whole organ, it can be life threatening. The first stage in scarring is inflammation. This means that even without an actual injury, chronic inflammatory processes (toxic substances, virus infections) can trigger scarring, which affects entire organs, such as the liver and lungs. The number of new cases (incidence rate) of cirrhosis of the liver (a special designation for liver fibrosis) is 250 per 100,000 people per year in industrial nations. Besides alcohol-related cirrhosis of the liver, the chronic hepatitis virus is the second most common cause in industrial nations, causing 20-25% of cases. In Africa, the hepatitis virus – predominantly Hepatitis C – is the most common, causing 90% of cases.

In order to stop fibrosis and local scarring, we need to be able to curb inflammation and intervene in collagen metabolism. Ideally we would need to be able to stop either collagen excretion or deposition around the producing cells. Wound healing studies conducted on conventional small laboratory animals such as rats and mice have to bear in mind that skin wounds in these animals heal through contraction rather than through scarring. Certain forms of scarring, such as keloids, appear to be specific to humans and cannot be perfectly reproduced in animal experiments. Liver fibrosis models are usually produced in rats by administering substances that are toxic to the liver, such as carbon tetrachloride. Such animal experiments are time consuming and complex.

Cell culture systems therefore lend themselves to pre-testing anti-fibrotics *in vitro*. Unfortunately, collagen deposition, i.e. the formation of a fibre skeleton around cells, is very inefficient in the standard culture medium. This is because the enzyme BMP-1, which trims the procollagen released into the culture medium to make collagen, works very slowly in an aqueous environment. Only collagen, not procollagen, can clump together to form insoluble fibres. BMP-1 is therefore a limiting factor in collagen matrix building *in vitro*. Most academic and pharmaceutical teams are still not aware of this. The standard culture medium is an artificial, highly aqueous environment in which cells are not usually found. In reality, the interiors of cells are filled with a large number of macromolecules, and the microenvironment of cells is dominated by macromolecules. There is little free water, either inside the cells or in their environment. This condition is called macromolecular crowding. We have been developing artificial macromolecular crowding for cell culture for 15 years (Chen et al., 2011). Adding macromolecules (usually sugar polymers) accelerates the activity of the procollagen-trimming enzyme BMP-1 and therefore results in rapid conversion of procollagen to collagen and thus to efficient collagen deposition *in vitro* for the first time. Other enzymes which chemically connect and therefore stabilise the collagen skeleton are also accelerated, as is the polymerisation of collagen molecules (fibril formation). Using macromolecular crowding we have created a smart culture system in which the entire scar cascade can be reproduced in a petri dish, and in which the relevant biochemical and enzymatic processes function efficiently.

We have successfully tested the Scar in a Jar system and in the case of anti-fibrotic substances, we were able to prove beyond doubt to two different pharma companies which substances work and which do not (Chen et al., 2009). If one of the companies had applied macromolecular crowding *in vitro*, the ineffectiveness of the substance would have become

clearer and would have made subsequent animal testing unnecessary. In retrospect, it became clear why the results of the animal experiments produced unclear results at considerable cost. The Scar in a Jar thus fills a test gap in the development of anti-fibrotics and has been adopted by industry: since 2011 it has been successfully used by GlaxoSmithKline for *in vitro* testing of substances to treat pulmonary fibrosis.

References

Chen, C., Peng, Y., Wang, Z. et al. (2009). The Scar-in-a-Jar: Studying antifibrotic lead compounds from the epigenetic to extracellular level in a single well. *Br J Pharmacol 158*, 1196-1209. doi:10.1111/j.1476-5381.2009.00387.x

Chen, C. Z. C., Loe, F., Blocki, A. et al. (2011). Applying macromolecular crowding to enhance extracellular matrix deposition and its remodeling in vitro for tissue engineering and cell-based therapies. *Adv Drug Deliv Rev 63*, 277-290. doi:10.1016/j.addr.2011.03.003

Microphysiological systems in translational research – applications and perspectives

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The role of the microbiome in the function of the human gut has attracted a great deal of attention in recent years. It is becoming increasingly clear that a physiological gut-microbiome interaction is essential to good health. If the composition of the microbiome (defined as the full array of microorganisms that live on and in humans) becomes physiologically unfavourable or even dominated by pathogenic bacteria (dysbiosis), this has a significant impact on the development and progression of diseases such as inflammatory bowel disease, organ failure in critically ill patients and sepsis.

A disruption of epithelial and endothelial intestinal barrier function is a typical pathological change in acute sepsis. Different mechanisms are currently under discussion to explain this. It is assumed that both signalling processes of an excessive immune response and direct interaction of the microbial pathogens with the epithelium and endothelium cells lead to a disruption of the barrier function. As a result of the subsequent systemic inflammatory responses and infections, multiple organ dysfunctions occur, with the liver being one of the first organs affected due to its direct connection to the gut. The liver is home to around 80% of the body's macrophages. Circulating monocytes constantly patrol inside the hepatic vascular system for pathogen-associated molecular patterns (PAMPs). Once PAMPs are detected, they are migrated into the liver tissue. To avoid adverse immune responses, endotoxins from the microbiome are tolerated by the liver within defined limits under physiological conditions. However, efficient defence against infection requires strict regulation of the inflammatory response on the one hand, and immunotolerance on the other. In this respect, macrophage activation is a central aspect as these cells are able to mediate both inflammatory responses to control bacterial infections, and tolerance of physiological endotoxin concentrations.

Due to the limitations of currently available *in vitro* methods, we often revert to animal models to study the physiology of gut-liver interaction and how this is deregulated in cases of infection and sepsis. Important anatomical and genetic similarities between mice and humans, as well as low husbandry costs, high reproduction rates and a short lifecycle compared with other mammals, have led to the mouse model being widely used in inflammation research in recent years. However, on account of obvious differences in diet, habitat and body size between mice and humans, there are still significant differences between the species. For example, species-specific metabolic requirements have resulted in significant evolutionary differences in the structure and microanatomy of the gut, and the function and composition of the immune system.

These significant limitations have led us to develop microphysiological models of the human gut and liver, with which we can describe the complex interactions in detail between the two organs on a molecular and cellular level in physiologically relevant conditions *in vitro*. The organ models are already routinely used as a central tool to study the pathophysiology of organ failure in sepsis, and to develop new therapeutic approaches for patients. At the same time, they are paving the way for the permanent replacement of animal testing in research at the Centre for Sepsis Control and Care at Jena University Hospital and in interregional research projects with academic and industrial research partners. I look forward to presenting this organ model and the associated organ-on-a-chip in more detail during my talk.

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