



Presentations of the 9<sup>th</sup> Conference on  
Animal Testing

**“Alternative methods  
—  
where now?”**



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**Speakers at the 9<sup>th</sup> SAP Conference on Animal Testing**  
**“Alternative methods – where now?”**,  
held at Hotel Arte, Olten, Switzerland, on 3<sup>rd</sup> March 2016

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## Introduction

Dr Julika Fitzi-Rathgen MLaw, Specialist unit animal experiments, Swiss Animal Protection SAP, at the 9th SAP Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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 9<sup>th</sup> 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**

Dr Mardas Daneshian, CEO, CAAT-Europe, University of Constance, Germany, at the 9<sup>th</sup> SAP Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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 bio-materials (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

Prof Rolf Zeller, Department of Biomedicine, University of Basel, University of Basel, Vice President of the "Research for Life" Association, Basel, at the 9<sup>th</sup> Conference on Animal Testing, "Alternative Methods – where now?" on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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

Dr Tina Buerki-Thurnherr, Empa, Swiss Federal Laboratories of Materials Testing and Technology, Laboratory for Particles - Biology Interactions, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland, at the 9<sup>th</sup> Conference on Animal Testing, "Alternative Methods – where now?" on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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?

Prof Olivier Guenat, Head of Organs-on-Chip Technologies, ARTORG Centre for Biomedical Engineering Research, University of Bern, Bern, at the 9<sup>th</sup> Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

## 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<sub>2</sub> 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 cells 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 en-

dothelial 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 breathe 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 200µm. 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 previ-

ously 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

Dr Kaspar Jörger, Head of Animal Welfare, Swiss Federal Food Safety and Veterinary Office FSVO, Bern, at the 9<sup>th</sup> Conference on Animal Testing, "Alternative Methods – where now?" on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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 an-

imal 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?

Dr Peter Bossard, Vice President, 3R Research Foundation, Münsingen, Switzerland, at the 9<sup>th</sup> Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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!

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### “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, see website at:

[http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch\\_id=20103576](http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20103576)

On 17<sup>th</sup> 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.*** See website at:

[http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch\\_id=20123660](http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20123660)

### Profile and track record of the 3R Research Foundation <http://www.forschung3r.ch/>

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 concentrated from the very beginning on pragmatically-oriented ra-



ther 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:

[http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch\\_id=20143683](http://www.parlament.ch/d/suche/seiten/geschaefte.aspx?gesch_id=20143683)

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 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 subdivides 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 Na-

tional 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.

P. Bossard, 1 Feb. 2016\*\*\*

### **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.

P. Bossard, 1 Feb. 2016

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 19<sup>th</sup> 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 perceptible in relation to the status of science at that particular time. For example, an answer could be found, even in the 19<sup>th</sup> century, for the question about how much arsenic had to be swallowed by a mouse weighing 100g to cause its death. Since then, however, our knowledge has improved enormously. Even by the middle of the 20<sup>th</sup> 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.

P. Bossard, 31 Jan. 2016



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

Maya Graf, Member of the National Council for the Greens in the Canton of Basel Land, Member of Switzerland's Commission for Science, Education and Culture, at the 9<sup>th</sup> Conference on Animal Testing, "Alternative Methods – where now?" on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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 25<sup>th</sup> 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

Thomas Cueni, Secretary General of Interpharma, Basel, at the 9th Conference on Animal Testing, "Alternative Methods – where now?" on 3rd March 2016 in Olten, Switzerland

### 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 formulation 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 Switzer-

land, 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 se-

lection 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

Prof Michael O. Hengartner, Rector, University of Zurich, Zurich, at the 9<sup>th</sup> Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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

Dr Stefanie Schindler, Co-Executive Director, Animalfree Research, Bern, at the 9<sup>th</sup> Conference on Animal Testing, “Alternative Methods – where now?” on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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

Dr Hans-Ulrich Huber, Chief Executive Officer, Swiss Animal Protection SAP, at the 9<sup>th</sup> Conference on Animal Testing, "Alternative Methods – where now?" on 3<sup>rd</sup> March 2016 in Olten, Switzerland

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.

