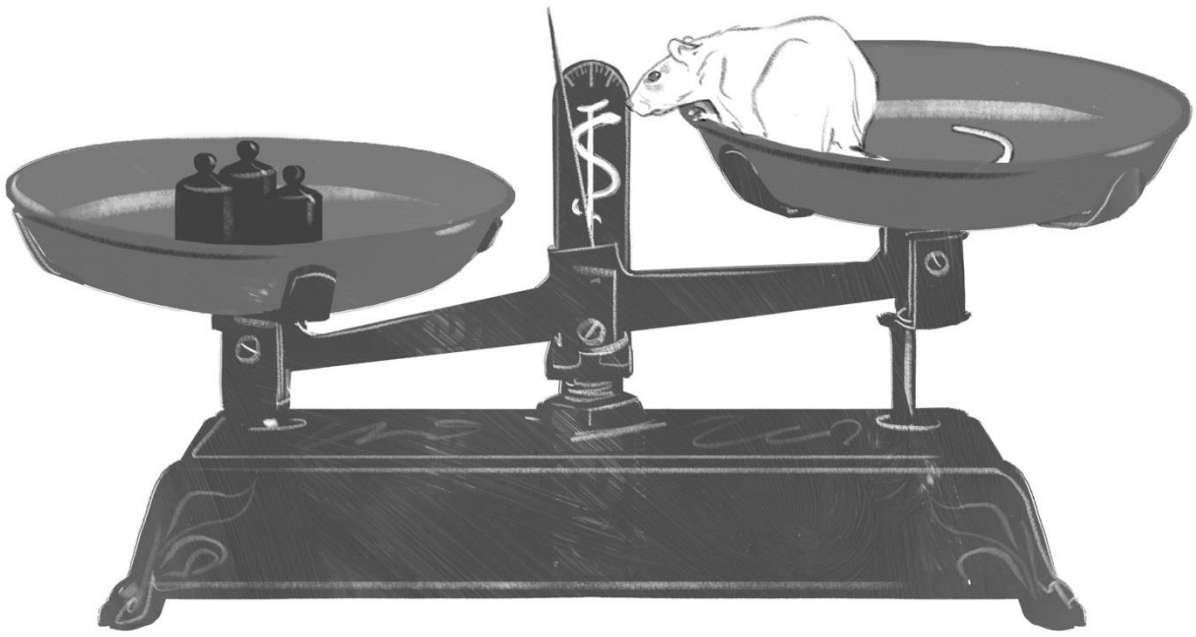
Presentations of the 10th Conference
on Animal Testing

"The Quality and Validity of Animal Experiments"



Hotel Arte Conference Centre, Olten, Switzerland
9th May 2017

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Speakers at the 10th SAP Conference on Animal Testing
"The Quality and Validity of Animal Experiments",
held at Hotel Arte, Olten, Switzerland, on 9th May 2017

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Introduction

Dr Julika Fitzi-Rathgen MLaw, Department of Animal Protection & Genetic Engineering, Swiss Animal Protection SAP, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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

Prof Hanno Würbel, Head of Animal Welfare Department, Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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 publica-

tions 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.

Vogt, L., Reichlin, T.S., Nathues, C., Würbel, H. 2016. Authorization of animal experiments in Switzerland is based on confidence rather than evidence of scientific rigor, PLOS Biology, 14(12), e2000598. Doi: 10.1371/journal.pbio.2000598

Reichlin, T.S., Vogt, L., Würbel, H. 2016. The researchers' view - Survey on the design, conduct, and reporting of in vivo research, PLOS ONE, 11(12), e0165999. Doi: 10.1371/journal.pone.0165999

Würbel, H. 2017. More than 3Rs: The importance of scientific validity for harm-benefit analysis of animal research. Lab Animals, 46(4):164-166. Doi: 10.1038/laban.1220

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

Prof Andrew Knight, Professor of Animal Welfare and Ethics, Director Centre for Animal Welfare, University of Winchester, UK, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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

Prof Anne Beuter, Emeritus Professor of Neuroscience, Bordeaux Polytechnic Institute, France, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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 [1] and Benazzouz et al., 2016 [2]), 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) [3], 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 [6]).

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.

(I) Historical, logical and mechanistic perspectives

From a historical viewpoint the articles by Verdier et al. (2015) [1] and Benazzouz et al. (2016) [2], 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) [2] 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 n*ucleus 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) [11] 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) [12] in France and Jasper (1966) [13] 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 [16], Bailey and Taylor (2016) [17] and Bailey (2015, 2015), [18-19]).

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 neuroanatomy, 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) [20], Benabid and Torres (2012) [21]). Finally, in the third part, we will see from the work of Frank (2005 [22]) 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" [23]. 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, Munoz et al. (2016) [24] 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) [25]), nor the observed variations in the clinical phenotypes, nor the fluctuating nature of the symptoms (Blesa and Prezedborski, 2014 [26]). 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 (Molnar et al., 2016 [28]).

Furthermore, the research work carried out by Goulas et al. (2014) [29], 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.

(II) 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) [5] 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 [32]).

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 to write in 2014 [34] that the imaging processes applied to the human nervous system (*neuroimaging*) would belong in the realm of "Big Data" in future"[34] (P. 2).

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 [36]). We have known about these rhythms for about 100 years (Little and Bestmann, 2015 [37]), 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) [38] 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 [37]). In addition, neuronal networks are now described on more spatial, chronological and topological levels (Betzel and Bessett [39]).

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 [41] (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 [42]).

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 neurostimulation 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 neuromodulation in Parkinson's disease". (Modolo et al., 2015 [42] (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?

(III) Discussion and conclusions

The economist Joshua Frank (2005) [22] 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 wrote in 2014 [49] 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 (P.417) [49]". 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 [50]).

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) [49]). As Duhigg (2013) [53] 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 (Duhigg, 2013 [53]). According to Frank (2005) [22] 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" (Pages 562-563) [22]. 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 [22]). 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 academic, 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.

The licensing procedure for animal experiments in Switzerland

Dr Kaspar Jörger, Head of Animal Welfare, Swiss Federal Food Safety and Veterinary Office FSVO, Bern, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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

What is an animal experiment?

	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 behavioural biology	• For teaching, education and training purposes
	Education/teaching (universities, secondary schools, laboratories)	• For teaching, education and training purposes

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

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

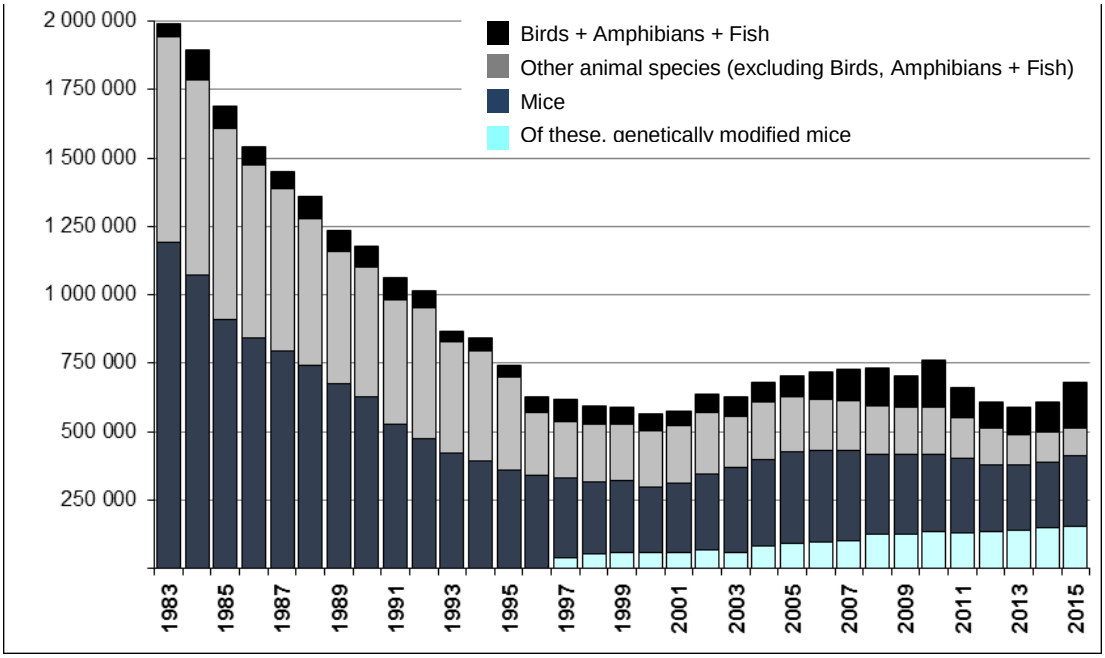
Reduce and Refine

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

The changes in animal experiments



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

Levels of severity

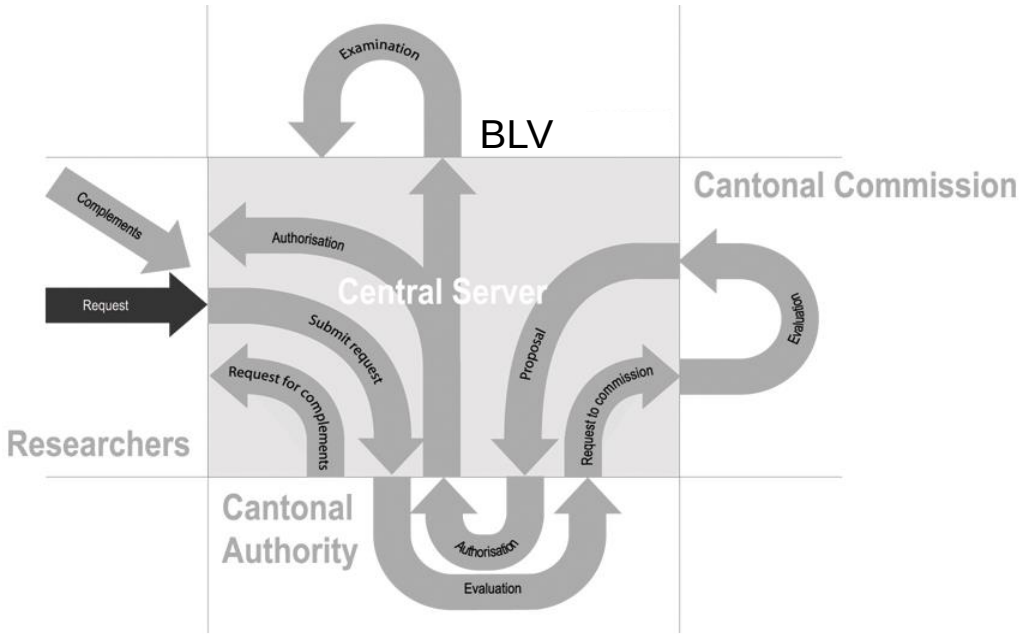
	Examples
0	Improvement in the husbandry systems for chickens Video recording for studies on the dehorning of kid goats Removal of organs for in vitro experiments
1	Experiments carried out under full anaesthetic, where the animal is eu-thanised while still under anaesthetic Vaccination, euthanasia and 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



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)

Würbel Study and the application for animal testing (Form-A)

The Würbel Study involves the following clauses of the application:

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

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)**

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.

Optimisation of education, training and ongoing training systems

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

lic. jur. Vanessa Gerritsen, Deputy Executive Director of TIR and Member of the Canton of Zurich’s Cantonal Committee on Animal Testing, Zurich, at the 10th SAP Conference on Animal Testing, “The Quality and Validity of Animal Experiments”, held on 9th May 2017 in Olten, Switzerland

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 – If the quality of animal experiments is being criticised, it is high time to focus on the promotion, development and implementation of alternative methods

Maya Graf, Member of Switzerland's National Council for the Green Party and Member of the Science and Culture Commission WBK-NR, Bern, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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 undertaking 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

Dr Jens Kelm, Director of Technology and Co-Founder of InSphero AG, Schlieren, Switzerland, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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

Dr Inge Herrmann (represented by Dr Nils Bohmer), Research Group Leader, EMPA (the Swiss Federal materials testing and research establishment, St. Gallen, Switzerland, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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) [1]. Despite promising results *in-vitro* and *in-vivo* it is not easy to apply one of these processes in hospital [1–3]. 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 [4]. We investigate selected *in-vitro*, *ex-vivo* and *in-vivo* models and their advantages and disadvantages and show some procedural changes and medium optimisations (Diagram 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.

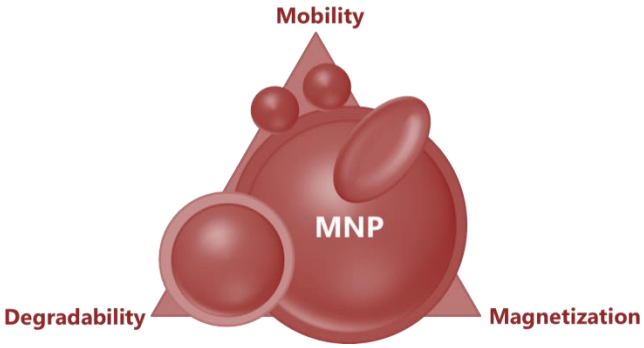


***Diagram 1**: Optimisation of magnetic agents on the basis of mobility, magnetisation and degradability.*

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

Prof Thorsten Buch, Director of the Institute for Laboratory Animal Science at the University of Zurich, Zurich, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

Working with animals from the simplest single-cell organisms all the way to 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 parameters 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 Swiss3RNetwork and via Twitter. Collaboration between the Swiss3RNetwork 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 re-

search 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?

Prof Margarete Arras, University of Zurich, Division of Surgical Research, Department of Surgery, University Hospital of Zurich, Zurich, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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)

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 classification. 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 well-being 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

Prof Christian Leumann, Rector of the University of Bern, President of the Research Delegation, Project Director for Concept Development for 3RCC (National 3R Competence Centre), swissuniversities, Bern, at the 10th SAP Conference on Animal Testing, "The Quality and Validity of Animal Experiments", held on 9th May 2017 in Olten, Switzerland

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 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

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

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.

The 3RCC in overview	
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.

Notes