



Good science with less animal experimentation

Replace

Reduce

Refine





For the sake of both science and laboratory animals

Non-animal tests cannot yet replace animal tests completely. Despite great efforts made in the past, some animal testing is still essential. Scientific questions exist that can only be answered by performing animal experiments. And some animal-based tests are required by law, for example for the toxicological testing of chemicals.

This is where the work of the 3R Research Foundation takes up: It is its aim to replace animal experiments whenever possible by methods that do not involve laboratory animals (Replacement), to reduce to a minimum the number of animals used (Reduction), and to refine necessary tests and the handling of the animals so that their pain and distress is minimised (Refinement). Together, these 3Rs are important for the welfare of animals, but the same principles also lead to better scientific results. The 3R Research Foundation supports the goals of the 3Rs by funding 3R-oriented research.

Since its establishment twenty years ago, the Foundation has demonstrated what can be accomplished when alleged opponents come together and follow common goals through concrete projects: In an unparalleled way the Foundation brings together industry, the government, academia, and animal welfare organisations.

The balance sheet is impressive: Over the last twenty years the Foundation has awarded funds for over 100 research projects, totalling a sum of 14 million Swiss Francs. Up until now this research has led to numerous publications that reveal the creativity of scientists in advancing the 3Rs in animal testing. Thanks to the Internet, the publications are accessible worldwide.

Nevertheless, a lot remains to be done. This is also shown by the fact that the number of animal experiments is on the rise again, after having decreased for twenty years in a row. Also in the future, the 3R Research Foundation will therefore continue to fund research projects that make a progress towards replacing animals and reducing the number and the distress of the animals used. For the sake of both animal welfare and science.

A handwritten signature in black ink, which appears to read 'Christine Egerszegi-Obrist'.

Christine Egerszegi-Obrist, Member of the Swiss National Council, Vice-Chairwoman of the 3R Research Foundation

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This brochure marks the 20th anniversary of the 3R Research Foundation.

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The challenging way forward

In the past decades, the number of research animals used in Switzerland decreased by 80 per cent. A significant part of this success can be attributed to the application of the so-called 3Rs principle. Although a lot has been achieved, there is still a considerable need for progress and improvement.

The subject of animal testing in research confronts most people with a dilemma. On the one hand, many people support the performance of basic research, the development of effective medications and warranty of the safety of chemicals. On the other hand, however, no one wants this to imply that animals must suffer fear, distress, and pain. This situation therefore leads to a number of questions: Does research really require as many animals? Can animal testing be conducted in a way that the animals do not feel distress or pain? Can testing be done without animals? These are exactly the three questions that lead to the 3Rs principle. This brochure explains the 3Rs and provides examples for how they are applied.

From 1983 to 2000, the number of research animals in Switzerland decreased from approximately two million to half a million. A decrease by almost 80 per cent. The 3Rs made a significant contribution to this success. But there are no grounds

to be satisfied with this achievement: Since 2001, there has been a slight increase in the number of laboratory animals used both in Switzerland and in Europe. This increase is most likely to result from the increase in biomedical research work performed at universities and in pharmaceutical industry, but also from more stringent safety regulations for medications and chemicals.

A vision difficult to achieve

Experts believe that a further decrease in the number of research animals used and especially a further minimisation of pain and distress still remain possible, but will be more difficult to achieve than in the past. The reason for this is that the more obvious 3R solutions have already been put into practice. For example, in the area of toxicity testing – the testing of the hazardousness of substances – in the last couple of years a number of ways to replace animal testing have been developed and put into practice in Europe.

“The vision of a world with no animal testing or at least the vision of experiments causing no distress to the animals has therefore become more difficult to achieve than before,” explains Hugo Wick, chairman of the 3R Research Foundation. “However, this makes it even more important to firmly anchor the 3Rs principle in basic research.”

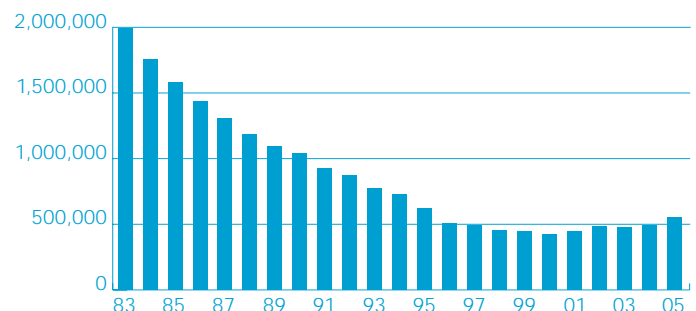
Animal experimentation: What does the Animal Protection Act say?

Animal experiments are defined as any procedure involving the use of animals performed with the goal (1) to verify scientific hypotheses, (2) to observe the effects of a particular procedure on the animal, (3) to test a substance (with exceptions), (4) to obtain or test cells, organs, or body fluids, (5) to meet the purposes of teaching and continuing education (Article 3 [c] of the Swiss Animal Protection Act). Persons who want to perform an experiment require its authorisation by the responsible cantonal authority (Article 18 [1] of the Swiss Animal Protection Act).

Statistics on laboratory animal use

In 2005, over 500,000 animals were used for scientific purposes in Switzerland. The majority of this research was conducted with the aim to improve human health, first of all in basic research (33 per cent of all research animals), and then in pharmacological research (52 per cent). Before a new product or medication may be put on the market, the respective producers are legally required to perform safety and quality controls using laboratory animals (10 per cent). Nine out of ten research animals are rodents (mice and rats). Approximately 4 per cent of the animals are subjected to severe distress (Severity Grade 3) during the experiment.

Animal experimentation in Switzerland
(The number of animals used from 1983 to 2005)



Animal experimentation requiring a license



It remains possible to further reduce the number of animals used in research, but this goal is more difficult to achieve today than it was in the past. For this reason it is all the more essential that the 3Rs principle becomes firmly established in basic research.

The two pioneers of the 3Rs principle: William Russell and Rex Burch



The 3Rs principle

The concept on the humane use of laboratory animals that the British scientists William Russell and Rex Burch proclaimed in 1959 may seem self-evident today. Nevertheless, they were pioneers in their day. It was their vision to strive for a humane relationship between people and animals. "Humane science is good science, and the best way to achieve that is rigorous application of the 3Rs," Rex Burch once said. It is against this background that Russell and Burch developed the 3Rs principle (Replacement, Reduction, Refinement).

The 3Rs describe in a nutshell what needs to be observed when planning an animal experiment. The commandment of Replacement demands that researchers and licensing authorities question the meaningfulness of the experiment. They are accountable for ensuring that the research is truly justifiable and that it can not be replaced by techniques that do not use living animals.

If an animal experiment is necessary and indispensable in accordance with the Swiss Animal Protection Act, the second commandment of the 3Rs, Reduction, requests to keep the number of animals used to an absolute minimum. The third commandment, Refinement, requires that those animals that do end up being used in an experiment should be inflicted with the least possible pain or distress.

It took some time for the 3Rs principle to find broader application. The change came about in the late 1970s not least due to pressure from animal protection organisations. Today, while the 3Rs concept is not yet taken for granted in all industrialised countries, widespread acceptance is growing.

The dilemma of animal testing

In principle, everybody is against animal experiments, just as all people expect the medications they take to be safe and the chemicals they come into contact with to be harmless. This is not possible without animal testing. What is the best way to deal with this dilemma?

A person's attitude towards animal testing can be categorical or pragmatic. Radical animal welfare activists reject animal testing categorically, because they consider animals to have equal rights to humans. The pragmatic view, however, places human welfare above the suffering of animals, which makes the use of animals in research justifiable. This second approach is laid down in current legislation. However, scientists using animals in research are required to weigh the distress imposed on the animals against the expected gain in knowledge. If this assessment leads to the conclusion that the benefit of the experiment outweighs the cost to the animal, the researcher can justify the experiment.

No animal testing for cosmetics

The greater the distress inflicted upon the laboratory animals used and the greater the number of animals required for a research project, the greater the expected benefit of the experiment has to be. For

this reason, no animal testing is conducted in Switzerland for the testing of, for instance, cosmetics. Lipstick and the like are considered to be luxury goods, and using cosmetic products does not represent an essential need to humans. It is more difficult to judge basic research, since researchers can not make any promises about the information that will be gained. However – despite great progress in the area of research on alternatives to animal testing – medical progress often-times continues to be achieved only at the cost of animal experimentation. Therefore one must set the goal to ensure that each individual laboratory animal suffers as little as possible and that the numbers of animals required for testing are reduced as far as possible. The right taken by humans to use animals in research is coupled with the obligation to conduct animal testing only on a justified case-by-case basis after a careful ethical balancing of the costs and benefits of the experiment.

Ethical considerations such as these are not binding in all countries. The reason for this is that people's attitudes towards animals are influenced by culture, religion, and education. Other cultures come to different conclusions on the same issues regarding animal experimentation.

Ethical guidelines

A number of organisations, universities, and pharmaceutical companies require their members and employees to adhere to ethical guidelines when conducting animal experiments.

Ethical guidelines have been developed, amongst others, jointly by the Swiss Academy of Sciences and the Swiss Academy of Medical Sciences. Those guidelines explicitly refer to the 3Rs principle. In addition, a number of different committees deal with this topic, such as the Swiss Ethics Committee on Non-Human Gene Technology (ECNH/EKAH) and the Federal Committee on Animal Testing (EKTV).

www.samw.ch

A case of ethical ambivalence

"Animal testing confronts us with the question of where we humans stand in regard to animals. Already in antiquity, the ancient philosophers disagreed on the status of our fellow creatures: While some emphasised the intelligence of animals, others like Seneca believed that man is unique and therefore to be placed above animals. Later, many philosophers considered animals to be mere machines, thus widening the rift between man and

animals. The evolutionary theory diminished the rift, as it brought to the fore the relatedness among all living things.

Up until today researchers conducting animal testing are confronted with this conflict. However, they are no longer left alone to deal with the ethical acceptability of research using animals: The legal framework in Switzerland is one of the most rigorous worldwide and is based upon the philosophy of the 3Rs. This philosophy is necessary. Nevertheless, we must not fall prey to the illusion that thanks to the 3Rs, animal testing will someday become unnecessary. Science, as well, will not be able to dispose of the ambivalent ethical points once and for all."

Alexandre Mauron, University of Geneva



"We must not fall prey to the illusion that, through a lot of reduction, animal testing will someday become unnecessary."

A lot remains to be done

The past decades have brought upon considerable achievements in the area of animal protection. Nevertheless, animal protectionists are not running out of work. Particularly with regard to the implementation of the 3Rs principle in practice, there is still room for improvement.

Many animal protection organisations recognise that the situation of research animals in Switzerland has improved over the past 20 years. But they are equally convinced that sufficient progress has not yet been made. Further improvements are possible and necessary in many areas, for example in the care and housing of laboratory animals. In the majority of animal-based research projects, the housing conditions of the animals cause greater restrictions and thus greater distress for

the animal than the testing itself. Incorrect care and housing of animals can result in behavioural disorders and also in changes in the animals' immune systems.

Adapted to the needs of the animals

For this reason Swiss animal welfare groups demand that the housing and care of laboratory animals is adapted as far as possible to the animals' natural needs. This requires a precise knowledge of the housing requirements that are ap-

propriate for the health and the well-being of the respective species as well as a lot of empathy on the part of the responsible personnel. Regarding social species, it is essential to provide a social environment and to continuously monitor the compatibility of the animals in the group. For larger-sized animals exercise and space to move about are important. Care must always be taken to provide the appropriate physical environment regarding illumination, temperature, humidity, and noise level.

Alternatives to animal testing: Where do we stand today?

After 20 years of work of the 3R Research Foundation, it is time to take stock. More than half of all projects funded in this period contributed to a reduction of the number of animals used in the respective tests. With few exceptions, these projects belonged to the area of basic research. Nevertheless, the number of animals used in basic research continues to increase. Are we doing



“Fortunately, many innovative experimental approaches were funded that are suited to replace animal testing – in the long term.”

something wrong at the Foundation? Or would the number of animals used in research have increased far more pronouncedly without the projects funded by the 3R Research Foundation? We can only suppose that this is so. Should we target the research areas responsible for the increase in the number of research animals as the focus of our funding programme? Or should the Foundation simply promote more thinking? For example, reflections about the fact that no matter how many genetically modified mice are developed and used, valid disease models for humans can not be generated, because the genes

in mice and men do not necessarily contain the same information. And because in the end this means an invalid reduction to the genetic background that too greatly disregards the involvement of the psyche, environmental factors, and nutrition.

Fortunately, many innovative approaches were funded that are suited to replace animal testing – in the long term. Nevertheless, in many cases the implementation process requires improvement. For example, the Foundation actively supported the know-how for producing recombinant antibodies without any animal testing whatsoever. Scientifically, the problem has been solved. And yet, in the day-to-day laboratory routine, rabbits continue to be immunised; the method is not being used.

Franz Gruber, University of Constance, Germany, Secretary and Editor in Chief of ALTEX

Highlights of 20 years of funding research

Since the 3R Research Foundation was established in 1987, it has funded over 100 research projects. The goal of these projects was to develop methods that use fewer or no research animals and that improve the housing and care of laboratory animals. Many of the projects were crowned with success. The projects in-

involved the efforts of numerous scientists, some of which are pictured below.

On the following pages, nine successful projects are presented that were supported by the 3R Research Foundation. The projects range from the search for pain genes to ticks that feed on artificial membranes rather than on host animals.

Detailed information on these and other projects funded by the Foundation is available at www.forschung3R.ch (Funded Projects).



L to r: Felix Wolf, Cornell University (US); Paolo Cinelli, University of Zurich; Paul Flecknell, Newcastle University (UK); Hanno Würbel, Justus Liebig University Giessen (DE)

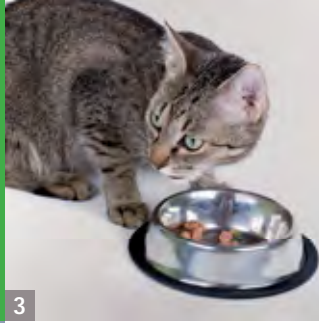
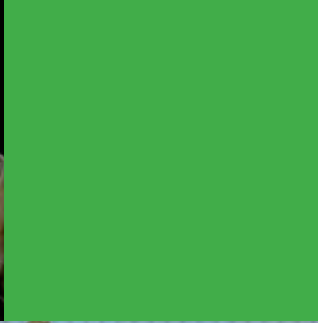
L to r: Eva Waiblinger, Swiss Animal Protection, Basel; Andrew Hemphill, University of Bern; Peter Brügger, Novartis Pharma AG, Basel; Paul Honegger, University of Lausanne

L to r: R. Geoff Richards, AO Foundation Davos; Marianne Geiser Kamber, University of Bern; Nicolau Beckmann, University of Basel/Novartis Pharma AG; Thomas Krober, University of Neuchâtel

Better boxes. When the cage contains more than nesting material, the animals show more meaningful activity and develop fewer stereotypic behaviours.



1



3



2



3

Recognising pain. In some animals it is difficult to determine if they are suffering. Rabbits, for example, sometimes mask signs of pain deliberately (left). In cats, a scruffy coat can indicate that the animal is experiencing pain (top).

Tracking down pain genes. Using DNA microarrays, the researchers examined 130 genes that are associated with pain and fear (photo shows microarray).

1 Minor changes, big effects

Making minor changes in the housing and care of laboratory animals can reduce or even prevent the development of stereotypic behaviours like constant chewing on the bars of the cage. These changes include adding "enrichment" factors to standard housing conditions that provide opportunities for activity and retreat, and also taking the social behaviour of the animals into consideration. Mongolian gerbils, for example, should not be separated from their families too early. In this study the researchers discovered that young gerbils should not be separated from their parents before a new litter of young siblings is born.

www.forschung3r.ch
Project number: 58-97

2 In search of pain genes

Researchers assume that certain genes increase or reduce their activity as soon as an organism experiences pain. For this reason they are looking to assess pain at the molecular level by identifying indicator genes. The identification and description of these genes might make the assessment and treatment of pain in laboratory animals much easier. Pain-relieving medications could be given to the animals at the right time and in the correct dose.

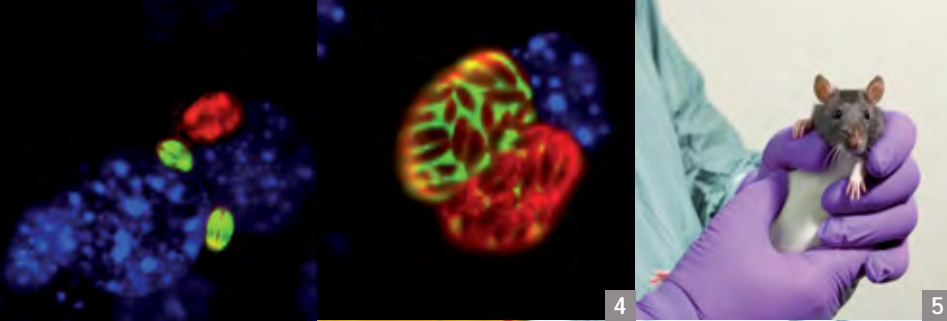
www.forschung3r.ch
Project number: 96-05

3 Recognising pain in laboratory animals

The welfare of animals in research is of central concern. Therefore it is important to recognise distress and pain already at an early stage. Only well-trained and experienced personnel can be expected to recognise these changes. However, the training of prospective research staff often fails because of a lack of effective illustrative training material. In this project a learning programme was developed and made available on the Internet. Using the programme, people can learn to recognise the behavioural signs of pain or distress in animals.

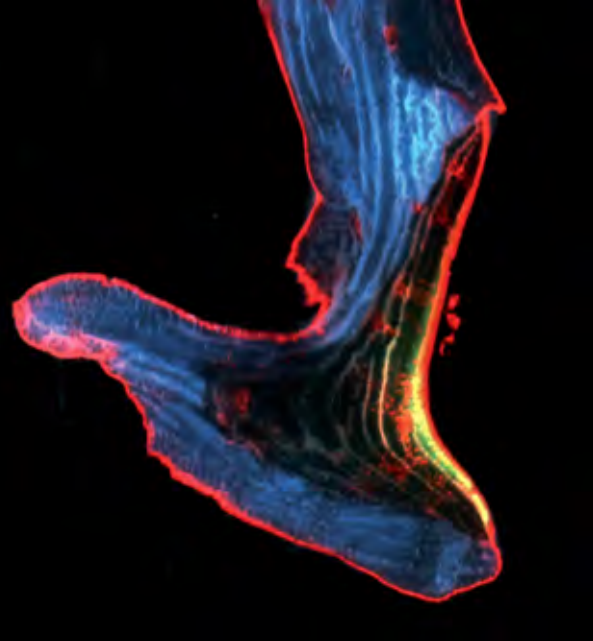
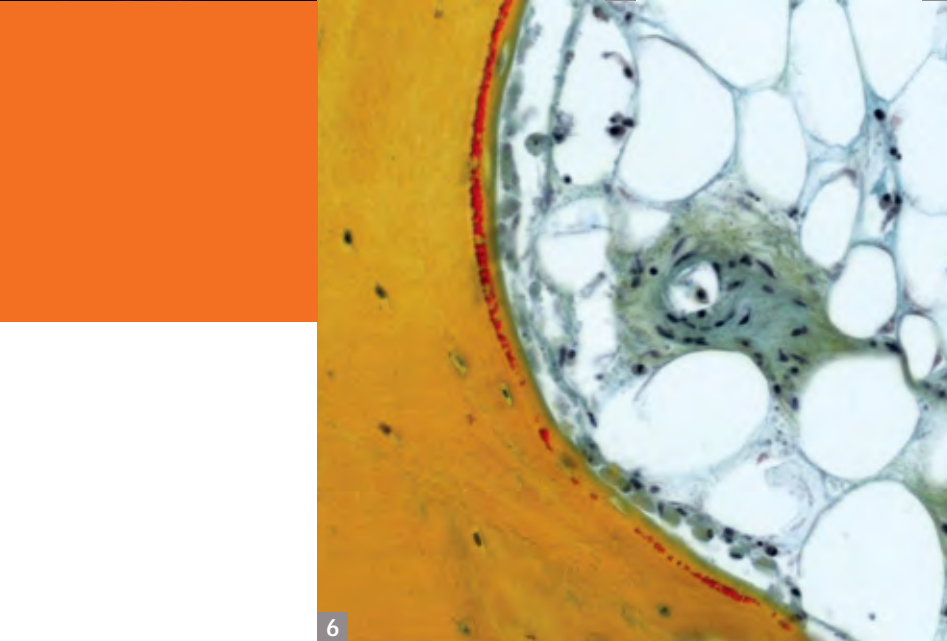
www.ahwla.org.uk
<http://3r-training.tierversuch.ch>

www.forschung3r.ch
Project numbers: 88-03, 71-00



Use of intestinal cells. Epizootic agents are investigated in cells instead of in the actual intermediate hosts, cats and dogs (parasites shown in red) (4).

Technology instead of rodents. When researchers work with mice and rats, they have to screen them for viral infections. In the past this screening was oftentimes performed using mice. Today, the PCR technique solves that problem (5).



Unburden sheep. Testing implants not in living sheep but by using bones of animals from the slaughterhouse.

4 Cells replace dogs and cats

The two parasites *Neospora caninum* (intermediate host: dog) and *Toxoplasma gondii* (intermediate host: cat) cause diseases in humans and animals. Research on these parasites is required in order to find effective medications or vaccines. Both parasites undergo a life cycle composed of three distinct stages. Thanks to the research projects, these stages of the parasites can now be cultured to some extent in vitro in intestinal cells. This means that the studies can be performed without the use of dogs or cats as intermediate hosts. Additionally, the scientists can also screen medications against the respective diseases in the cell cultures.

www.forschung3r.ch
Project numbers: 85-03, 72-00

5 Molecular biology replaces rodents

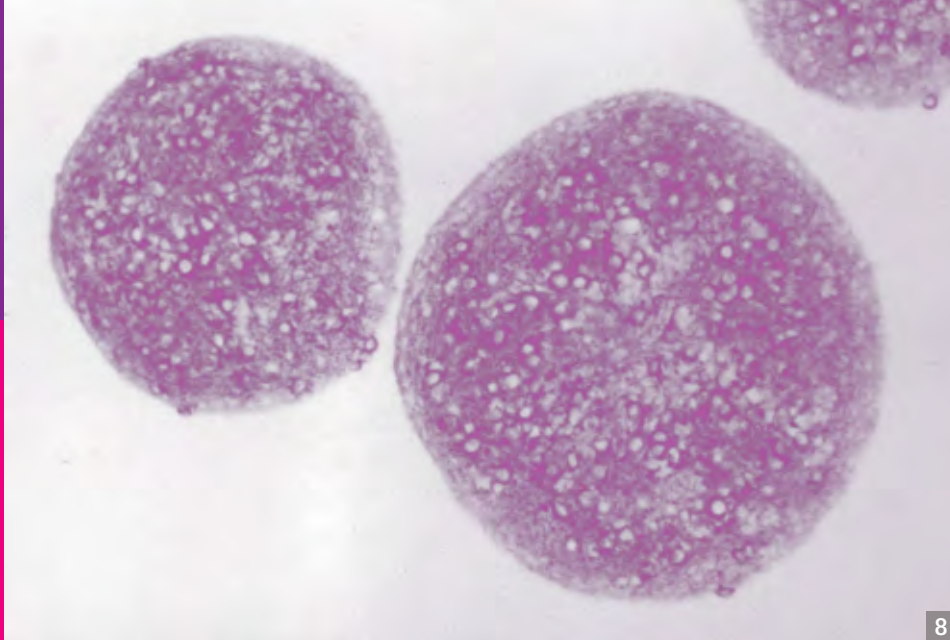
When researchers work with rodents, it is important that the rodents are pathogen free. The traditional test to screen rodent strains for viruses used numerous animals. Researchers in Zurich and New York have developed a replacement for the traditional mouse antibody production (MAP) test. Today the test can be conducted using molecular biological methods (PCR assay) – which require no animals. The PCR technique not only reduces the use of animals but is also cheaper, faster, and more reliable.

www.forschung3r.ch
Project number: 74-00

6 Testing implants using ex vivo bone core culture systems

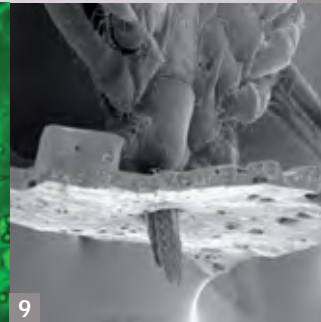
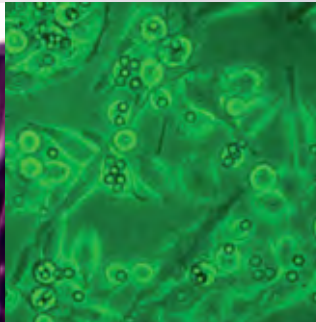
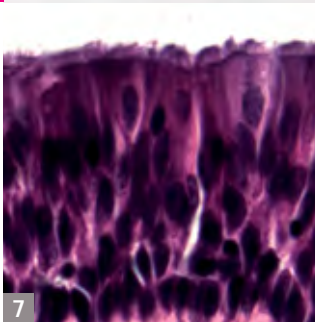
Implants that are used in the treatment of, for instance, bone fractures are mostly tested in sheep. However, an alternative exists: By using cultures of live bone cores, it is possible to obtain osteal tissue that largely retains the same functions as bones in the intact organism. This is achieved by periodically exerting a force on the cultured bone material. Processes in the bone can be studied more efficiently in this osteal tissue with the result that part of the testing of implants no longer needs to be performed in sheep. Some bone cores are taken from dead animals from the slaughterhouse; another source is human bone that is no longer required after hip operations.

www.forschung3r.ch
Project numbers: 86-03, 78-01



Reduced blood flow. With the use of brain cell cultures, researchers can investigate the mechanisms and the progress of ischemia.

8



Scantly studied particles. The effect of particles on the lungs can be studied in a cell culture system.

A tick penetrates into a membrane using its proboscis (left). Less thirsty ticks replete with blood (right).

7 Study particles in cell cultures, not in lungs

It has long been recognised that most respiratory diseases are caused by the inhalation of particles. However, little is known as yet about the health effects of nanoparticles, in particular. Testing the harmful effects of particles in a living system is very stressful for research animals, time-consuming, and costly. In this project the researchers developed a three-dimensional primary cell culture system in order to study particle-lung interaction. In a special chamber system, specific cells that react directly to inhaled particles (such as epithelial cells) are exposed to particles, and the effect of the particles on the cells is measured. The use of this method can reduce animal experiments where inhalation is required to identify possible lung damage by certain substances.

www.forschung3r.ch
Project number: 89-03

8 Using brain cell cultures to study brain damage

Inadequate blood flow, or ischemia, leads to nerve cell damage in the brain within a short period of time. A large part of current brain ischemia research is conducted using animals, and it can cause the animals a lot of distress. Scientists have developed a three-dimensional brain cell culture system that resembles brain tissue in many of its characteristics. This means that various aspects of ischemia research can now be investigated in the test tube instead of in animals. Due to its significance, this in vitro method is also being evaluated by the EU ACuteTox Project as a model for identifying neurotoxic chemicals without laboratory animals.

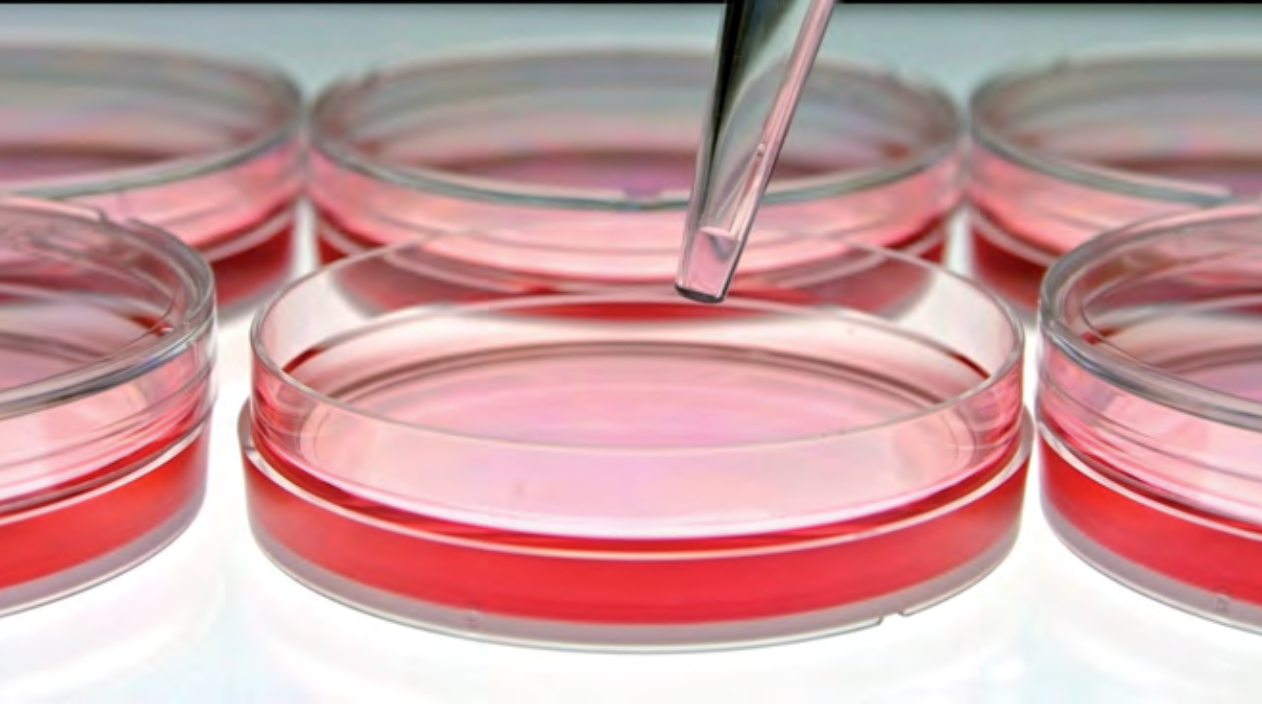
www.forschung3r.ch
Project number: 64-97

9 An artificial feeding system for ticks

Animal experiments are required to develop repellents against ticks. Host animals are necessary to allow the ticks to feed on their blood. Scientists have developed artificial feeding membranes that simulate the skin of host animals. The feeding medium (blood) is located beneath the membrane, and test products can be added to it. Female ticks feed until replete with blood and then produce eggs, from which larvae hatch. The in vitro system can be automated and can be used to test new anti-tick products. The system has many advantages as compared to traditional testing methods, and a large number of host animals can be saved.

www.forschung3r.ch
Project number: 79-01

Cell culture in a Petri dish. Sometimes it is possible to refrain from performing an animal experiment by reverting to cultured cells.



Replace: Non-animal test methods have their limitations

Replacing animal testing with a method that does not use animals is the best solution, but it is not always possible. In the past, cells have proved very useful, but these systems have their limitations, too.

Replacement means the use of methods other than conducting experiments on animals. This is not a simple task, because a single 3Rs method rarely serves to directly replace a specific animal experiment. However, by combining one or more 3Rs methods, one can perhaps already yield sufficient information to make the animal experiment unnecessary.

Cells have proved very useful. For instance, it has become possible to use layers of cells to build a kind of artificial

skin that can be used for the evaluation of the effect of possibly harmful substances. This is of interest to the cosmetics industry, for example. Nevertheless, research using cells, tissues, and isolated organs also has its limitations, since complex phenomena of the intact body can not be investigated. Or, stated more plainly: Cells neither experience fears nor have diarrhoea.

Replace: What does the Animal Protection Act say?

Based on the obligation to "limit to the indispensable extent", an experiment with animals must not be authorised if the goal of the experiment can be achieved by using methods without animal testing that are suitable according to the current state of knowledge. An animal experiment is particularly inadmissible if it causes the animals a disproportionate degree of pain, suffering, injury, or excessive fear in comparison to the expected gain in knowledge.

Art. 17 and Art. 19 (4) of the Swiss Animal Protection Act

No animal testing for cosmetic products in Switzerland

Until today, many Swiss are convinced that cosmetic products are tested on animals before they are put on the market. However in Switzerland, this has no longer been the case for several years. In the meantime, the European Union (EU) has also responded to this issue. As of September 2004, it banned all animal testing of finished cosmetic products in the EU. In Germany, Austria, the United Kingdom, and the Netherlands animal testing for cosmetics has been banned by national legislation since the late 1990s. Nowadays, most of the testing is conducted using cell cultures instead of animals. In the past years, the cosmetics industry has

invested 500 million Euro in alternative research. So far, the European Centre for the Validation of Alternative Methods (ECVAM) has validated nine alternative methods.

The next milestone will be the date of the 11th of March, 2009, when the EU will ban all animal testing of cosmetic ingredients within the EU, irrespective of the availability of non-animal tests. In addition, apart from a few exceptions, there will be a ban on the marketing of cosmetic products tested on animals from that day on. This new cosmetics EU Directive will affect 2,000 companies across Europe.

Blood cells save 500,000 rabbits every year

The 21st of March, 2006, was an important day for many laboratory rabbits, since that was the day that members of the ECVAM Scientific Advisory Committee (ESAC) issued a statement to the European Commission recommending five tests for the replacement of the rabbit pyrogen test. The pyrogen test is a safety test for the detection of contaminants in medical products. The new assays use cell cultures instead of live rabbits. Experts estimate that these new methods will save the lives of 200,000 rabbits in the EU and of half a million rabbits worldwide every year.

Pyrogens are substances that can induce fever in humans and even life-threatening shocks. Before medical products are put on the market, they therefore have to be tested for these undesired substances. For more than fifty years, pyrogen detection relied on the use of rabbits: In the rabbit in vivo test, a sample of the medical product was injected into the animals, and their body temperature measured and recorded. An increase in body temperature indicated pyrogen contamination.

The five assays endorsed by ESAC in 2006 can fully replace the rabbit pyrogen test. They offer a number of advantages: they take less time, are less costly, and show better sensitivity. All of the new tests are conducted using human blood cells in vitro.

www.forschung3r.ch
Project number: 52-96

Complete replacement of the Draize test foreseeable

The Draize test for eye irritation, which was developed in the 1940s, uses rabbits to test whether chemicals, cosmetics, or pharmaceutical products irritate the eye. The substance is applied to the eye of the animal and irritation is measured. Today, substances are started out at being evaluated in non-animal in vitro methods. Severely irritant and corrosive substances are not further tested on the eyes of rabbits. Only chemicals that have revealed no effects in non-animal tests are applied, in strongly diluted form, to the eyes of animals.

Nevertheless, this does not suffice. The search for a replacement test continues. In this context, a very promising approach being assessed is the artificial generation of the human cornea making use of the respective cell types. Such cultured cornea epithels are already available on the market. The reason the scientists are focusing on the cornea is that it is the first layer to come into contact with chemicals when they enter the eye.

Another method to replace such substance evaluations on the eyes of living animals makes use of the eyes of dead cattle and chickens from the abattoir. It is also in this area that progress has been made in recent years. Thus, it is to be hoped that these tests will be able to fully replace the Draize test in Europe in the foreseeable future, thereby finally completing the step from "reduce" to "replace".

10,000 rats spared

Since the mid-1980s, Novartis has been producing the active ingredient calcitonin. This substance provides patients with bone diseases, such as osteoporosis, with stronger bones, and in many cases it also reduces pain. However, before a new batch of the medication can be put on the market, tests have to be conducted to assure that the calcitonin in the chosen preparation is safe and effective. Until recently these tests were performed using rats.

Since the late 1990s, the Novartis Working Group on Biological Analysis has been searching for a method that uses cells instead of live laboratory animals for this evaluation. Finally, such a method was developed in cooperation with the University of Heidelberg and optimised by the Novartis scientists. For this method, cells are cultivated in vitro and then brought into contact with calcitonin. The hormone calcitonin binds to the cell membrane and triggers a response in the cells. Depending on the amount of calcitonin, the cells release a biochemical messenger (cAMP), which can be detected unequivocally and therefore serves as an indicator for the activity of calcitonin.

Using this method, which was validated by Novartis and approved by the U.S. Food and Drug Administration, 10,000 rats can be saved from calcitonin testing at Novartis alone.



Researchers place an anesthetised mouse into an MRI scanner. The MRI approach can reduce the number of animals used in a study by up to 90 per cent.

Reduce: As few as possible, as many as necessary

The second commandment of the 3Rs principle is Reduction. Today, many animal tests can be conducted using a fraction of the number of animals originally foreseen. In this context the use of new technologies, such as MRI or computer-based pharmaceutical development, are beneficial.

Reducing the number of animals used in research is a must, both ethically and economically. However, scientists have to ensure that the number of animals used is not reduced to a point where the data can no longer support a meaningful statistical analysis. Otherwise, the results lack validity, and the tests have to be repeated.

The main task in Reduction is to determine the optimum number of animals per group in a given animal test. Additionally, parallel investigations using non-animal test methods can generate information that can reduce the amount of animal testing needed.

3Rs not always in unison

Almost always, the 3Rs principle is described as a unit, but in practice there can be conflicts between the Rs, as the following example shows. In one experimental set-up, ten rats have to suffer pain and distress. A second possible research design requires 20 rats, but here the rats will suffer only little or no pain and distress. Which design should the scientist choose? This is a decision that has to be made on a case-by-case basis. Nevertheless, in principle, experts agree that the second variant is the better choice. Reducing the pain and distress of the

individual animal is more important than reducing the number of research animals.

Reduce: What does the Animal Protection Act say?

Experiments with animals, which cause the animal pain, suffering, injury, fear, or significantly disturb their general condition or which can disregard its dignity in any other way, must be limited to the indispensable extent.

Art. 17 of the Swiss Animal Protection Act

More information using fewer animals

Non-invasive methods exist for investigating research animals. Some examples are magnetic resonance imaging (MRI), positron emission tomography (PET), and computerised tomography (CT). The rapid progress that has been made in regard to these image-producing techniques in recent years can now also benefit research animals. For instance, scientists in the pharmaceutical industry follow the effects of drugs in an animal's body by continuously observing a single animal with the help of MRI, rather than killing different animals at different time points for their investigations (ref. interview on page 25).

In 2006, Nicolau Beckmann of the Novartis Institutes for BioMedical Research (NIBR) evaluated the applicability of MRI to evaluate pulmonary inflammations in small rodents. "Using MRI, a significant reduction in the number of animals used for experimentation was achieved. Depending on the application, the number of animals was 80 to 90 per cent lower than with conventional methods," explains Beckmann. Further advantages of MRI are minimal distress to the animals and the potential to standardise the test method. There are limitations, however: Currently, only a few MRI scanners exist that are adequate for the scanning of small rodents.

www.forschung3r.ch
Project number: 82-02

The LD50 test no longer exists

In November 2000, the OECD reached agreement to delete OECD Test Guideline 401, the LD50 Acute Toxicity Test. This test evaluated, which dose of a test substance would kill 50 per cent of the animals used within two weeks, the lethal dose for 50 per cent of the animals. Accordingly, the test served to determine acute toxicity, a measure of the immediate toxicity of a substance. The test was mainly used for determining the toxicity of chemical substances.

Even though the new alternative methods adopted by the OECD could not fully replace the acute toxicity test, instead of the 150 animals required in the 1970s, now only an average of 8.5 animals is necessary. This is achieved through a stepwise dosing of the animals, applying higher doses until symptoms appear. Once that is the case, the animals are killed.

In a next step, it is aimed to replace also these alternative methods currently approved by the OECD, with the final aim to test for acute toxicity without the use of animals. This is important in the face of the EU REACH regulation, the programme for the Registration, Evaluation, and Authorisation of Chemicals. Starting in 2008, 30,000 chemicals will be tested in the EU (see page 30). It is estimated that despite the non-animal test methods available today, several million additional laboratory animals will still be needed to conduct the large-scale testing and evaluation programme.

www.acutetox.org

Experiments with animals only after extensive test batteries

Today, computer methods also contribute towards reducing animal experiments. In drug discovery and development, for example, potential points of therapeutic intervention in the organism, the so-called drug targets, are identified in a first step. Once sufficient information is available – for instance regarding drug binding sites – compounds can be designed on the computer that fit into these binding sites. This process is called computer modelling. With the help of computers (the so-called in silico methods) and in automated tests (the so-called high-throughput screening), out of tens of thousands of substances, those compounds are identified that show the desired results in vitro (mostly in cell cultures). Only these few substances are then further investigated in studies using animals.

However, drugs do not only have to be effective, but they should also be safe. An initial prediction, whether a compound may possibly trigger side effects in the organism, can be made with the so-called QSAR methods (quantitative structure-activity relationship). The basic assumption in a QSAR model is that similar molecules will show similar harmful effects. Another method aiming in a related direction is presented on page 24 below.

For many years it has been thought that one day it would be possible to fully replace the testing of compounds by in silico methods – that is, by modelling the effects of compounds in the computer. However, this will not be possible in the foreseeable future, due to the complexity of the human body.



In company. Dogs should not be kept as single animals. Nowadays, systems for the housing of dog exist that make it possible to keep dogs in groups without influencing the results of the experiments.

Refine: Minimise the distress to the animals

Refinement encompasses a broad range of options that all aim at minimising the animals' discomfort. This includes housing social animals in groups and enriching their cages, for example by adding small shelters and pieces of wood.

Refinement encompasses all measures that make a contribution to reducing the distress, pain and suffering of the animals before, during, and after the experiment. However, Refinement is important not only

Refine: What does the Animal Protection Act say?

Pain, suffering, injury, or fear may only be inflicted on an animal to the extent that is unavoidable for the purpose of the experiment.

Art. 20 (1) of the Swiss Animal Protection Act

on animal welfare grounds, but also for scientific reasons, since distress of the animals can distort the results of the experiment.

Refinement involves the application of sterile surgical procedures, the proper use of analgesics, and provisions to handle the animals with the least possible stress during experimentation – but not only then. For it is not only the experiment as such that causes distress to the animals, but also the way in which the animals are kept and housed. Animals experience particularly high levels of distress if they are taken from wild habitats for use in research. For

this reason, animals that were bred for research purposes are used almost exclusively today. Distress is also caused by uncaring handling of the animals, cages without enrichments, and social isolation. Nowadays, these discomforts can be prevented.

In Switzerland, Refinement methods are promoted by the Swiss Laboratory Animal Science Association, in particular. Many of the members of the association are veterinarians.

www.sgv.unizh.ch

Enrichment: When the cage contains more than just straw

Laboratory mice and rats spend their lives in a cage. Different studies demonstrate that rodents housed in stimulus-poor cages had impaired brain development, and expressed stereotypies and anxious behavioural profiles. Through environmental enrichment by means of shelters, shredded paper, pieces of wood, and climbing opportunities, these developmental impairments can be reduced. Mice and rats want to explore their environment, build nests, and seek refuge when in danger. "A number of studies have shown that rodents make use of these structures, with the result that they become less anxious and develop fewer behavioural disorders," explains Hanno Würbel, Professor at the University of Giessen.

There were fears that enriched housing conditions would adversely affect the robustness of the data from animal research. A study conducted in 2004 by Würbel disproved these reservations. "Our study showed that enrichment by no means affects the significance of animal experiments," says Würbel.

www.forschung3r.ch
Project numbers: 77-01, 66-99

Social behaviour: Rodents live in groups

With the exception of hamsters, rodents – the animals used most frequently in animal experiments – live in groups. Therefore it is important that mice and rats are housed in groups and that group housing is only refrained from in exceptional cases. Rats, especially, are very social animals that groom each other and communicate in the group with scents and sounds.

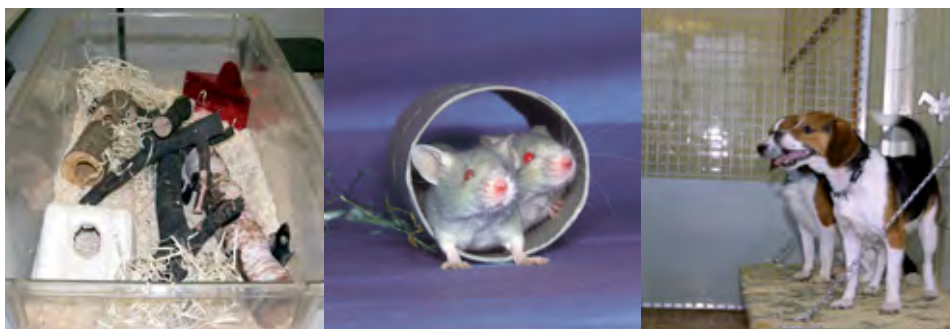
Within a group, rats and mice have a stable ranking order, which, however, can only be established if the group is not too large. Studies have revealed that the optimum group size for mice is four to eight animals and for rats three to four animals. If the groups are larger, there is increased fighting for rank order position. To prevent fighting among the animals, the group should be formed of young animals as early as possible and then left unchanged.

Dogs, too, should not be kept alone. Certified systems for housing dogs in groups exist, which still enable to track the necessary data (for example, food uptake) for each animal individually.

Humane endpoints: Prevent avoidable suffering

A humane endpoint is the earliest possible point in time, at which an experiment can be ended before the animal used has to experience too much distress or pain. It is defined as the point in time when the test can be stopped because its objectives have been met, but the animals have not yet suffered severe distress. Already prior to the testing, it is important to define, under which circumstances and by which means pain and distress in the individual animal will be avoided or terminated. These criteria are then assessed and recorded using so-called score sheets (such as rapid loss in body weight, difficult respiration, reduced grooming, or paralyses).

As a rule of thumb, animals have a similar capacity as humans to experience pain and distress. But this insight alone does not suffice. Trained personnel must be able to assess the animal's pain during experimentation. If animals must be killed during or after the experiment, this must be done as painlessly as possible and according to accepted methods that confirm with legal regulations (see page 18).



Less pain, more reliable test results

For some years now, animals have been given analgesics, if it was to be expected that they could suffer. This is of benefit to the animal, but it also benefits the research study as such, for tests using animals suffering pain can deliver worthless information.

“It is a common misperception that you can clearly see when an animal is in pain,” says Peter Maier, scientific advisor to the 3R Research Foundation. However, for instance, pain in mice and rats can be recognised only by trained personnel. “If a mouse shows abnormal behaviour, has an unnatural body posture, or loses body weight, then it has to be assumed that it is already experiencing very strong pain,” says Maier. In contrast, dogs show obvious signs of pain, and they can also alert people to their pain by the sounds that they make. In sheep it is not yet known how they show pain or enduring distress.

Some years ago it became regular practice to give laboratory animals pain relievers, if it was likely that they would suffer pain. Today, and especially for post-operative pain, the administration of analgesics is standard practice and is mostly also required by law. The advantages of pain relievers are obvious: Pain can affect the entire organism in an unpredictable manner to such an extent that a test can yield worthless results, and without the researcher noticing.

If the animals experience less pain, test results are more reliable.

For analgesics to be administered correctly, however, specialised knowledge is required. The signs of pain differ not only between animal species, but also depending on the type of surgical procedure or the organ causing pain. Researchers and care personnel must be able to recognise and assess how effective the pain relief measures are for the respective species. This requires knowledge regarding the way analgesics work, their duration of effectiveness, which parts of the organism they act upon, and the possible forms of administering the analgesic.

There are exceptions

Today, analgesics are withheld only in exceptional cases, such as in research trials investigating rheumatism, cancer, or similar diseases, because analgesics distort the results in those cases. In the course of such investigations, the trial is ended and the animal is killed as soon as the scientist recognises that the new drug does not have the desired effect.

Allowing the animals to become accustomed to the planned procedure through training is another important prerequisite to reduce the animals’ anxiety and pain. In this context, the care staff and their handling of the animals play an important role.

A good planning of a research project also includes the determination of criteria for discontinuation of the experiment. These are criteria that must already be defined in the application for a license to conduct the experiment and that lay down when the procedure must be terminated (humane endpoints).

The administration of analgesics admittedly also has disadvantages, for merely picking up an animal, even if it is done properly, can cause it anxiety. Therefore, the degree of pain reduction has to be weighed against the degree of additional stress. A stressed animal will experience greater distress during the procedure.





Knowledge required. It requires specialised knowledge to administer pain relievers in the correct dose at the right time, since animals do not all show pain in the same way.

Classification of animal experiments

No distress: Severity Grade 0

Interventions and manipulations in animals for experimental purposes as a result of which the animals experience no distress (no pain, suffering, or injury)

Examples: withdrawal of blood samples for diagnostic purposes in cows; the housing of rats in enriched environments for behavioural observations

Minor distress: Severity Grade 1

Interventions and manipulations in animals for experimental purposes which subject the animals to a brief episode of minor distress (pain or injury)

Examples: injection of a drug requiring the use of restraint; castration of male animals under anaesthesia

Moderate distress: Severity Grade 2

Interventions and manipulations in animals for experimental purposes which subject the animals to a brief episode of moderate distress, or a moderately long to long-lasting episode of minor stress (pain, suffering, or injury, extreme anxiety, or significant impairment of the general condition)

Examples: surgical treatment of a bone fracture on one leg that was purposely induced under anaesthesia; castration of female animals (under anaesthesia)

Severe distress: Severity Grade 3

Interventions and manipulations in animals for experimental purposes which cause the animals severe to very severe distress, or subject them to a moderately long to long-lasting episode of moderate distress

Examples: transplantations, potentially lethal infectious diseases



Sought-after genetically modified animals and animal models

Genetically modified animals are sought-after models in research, because they can serve to answer questions that can hardly be pursued by other methods. These animals provide a means to study, in living animals, what happens if, for example, the function of a particular gene is switched off.



Focus on transgenic mice. Genetically modified mice are popular models in research, because they allow scientists to study, in a living animal, the effects of the modification of a particular gene.

Oftentimes, diseases or physiological processes can not be studied in single, isolated cells. Alzheimer's disease, for instance, is a disease that affects not only individual cells but entire regions of the brain. In addition, the condition leads to behavioural changes that can not be investigated using cells. For this reason, scientists do not only make use of single "Alzheimer cells" but also of a so-called animal model. This animal model consists of genetically altered mice that develop a disease that, while not identical, is similar to the human Alzheimer's disease. Using these mice, researchers can gain new insight on the cause of Alzheimer's and can test new treatments.

Frequently, such animal models are produced with the help of genetic engineering methods. The advantage of experiments using genetically altered (transgenic) animals is that the intervention in the animal's genome takes place in a targeted manner and not randomly, as is the case in cross-breeding. In fact, singular genes can deliberately be switched off (knock-out animal) or inserted (knock-in animal) into the genome of these animals. Therefore transgenic animals allow observation of the effects of genetic modifications in living organisms.

Using genetically modified mice, significant advances were made in the investigations on the most common inherited disease in Western Europe, cystic fibrosis (CF). About one in 2,000 children are born with CF. These children produce a thick mucus in their lungs that can not be coughed up. A cure still does not exist, but thanks to transgenic mouse models of CF, much more is now known about this disease. This is the prerequisite for better therapies.

Several genes are usually involved

However, genetically engineered mice also do not enable scientists to answer all open questions. Many human diseases are not caused by a change in one individual gene, but rather by the interaction of several errant genes. This makes the analysis of diseases considerably more difficult. For this reason, opponents to animal testing point to the limited explanatory

power of research using genetically modified animals. Experts are of the opinion that the transferability of data from animals to humans varies greatly from case to case.

Nevertheless, transgenic animals are helpful and therefore sought-after models, particularly in basic research. This is one reason why the number of animals used in research is once again on the rise – especially since the mapping of the human genome. Whereas researchers now know all of the human genes, they still do not know the functions of many of them. Genetically altered animals can help find the answer to this question, because many human genes are found in mice in a similar form.

In spite of this, it is disadvantageous that it can take a very long time and require a large number of animals before a genet-

ically modified strain of mice is available for use: Its development can take up to several months or years. In addition, animal welfarists point to the suffering of the research animals that can be caused by the genetic alterations.

Genetically modified? Transgenic?

Genetically modified organisms are organisms, whose genetic material has been altered in a targeted manner with the help of genetic engineering techniques. The narrower term "transgenic" describes a similar condition: Transgenic animals are organisms, in which an existing gene has been switched off or in which foreign DNA has been inserted into the cells. In the year 2005 in Switzerland, 94,000 genetically modified mice were used in experimental procedures. Twelve per cent of these animals were subjected to moderate to severe distress.

Laboratory animals benefit from new methods

Classical animal models from genetically modified animals have a number of disadvantages. Oftentimes, they are not flexible, and the altered genes are active during the wrong developmental stages or in the wrong organs. This can result in incorrect conclusions drawn from the studies.

Switch on and switch off only when needed

Therefore systems are now increasingly being used that allow an "external" control of the activity of the genes. Examples for this are the Tet systems: The altered genes can be switched on and off at a desired point in time by introducing or withdrawing the antibiotic tetracycline.

Switch on and switch off only where needed

Classical knock-out mice already carry a genetically engineered genetic defect in all of their organs at the time of their birth. This can result in undesired disorders during the development of the animals. For this reason, knock-out mice are now increasingly being produced, in which the genetic defect is limited to one type of cell or one particular organ. This can be achieved with the Cre-lox system, for example.

Some of these new animal models can be acquired from internationally available collections. Therefore they do not have to be produced anew each time. Kurt Bürki, Professor at the University of Zurich and specialist in transgenic animal models, says: "These new systems are much more precise than the conventional ones. The experimental results are not distorted

by undesired side effects and are therefore of better quality. At the same time, fewer health problems are to be expected."

Another method, called RNA interference (RNAi), has the potential to make a contribution towards having to produce less genetically modified mice for animal testing. Using the RNAi method, genes can be turned off in normal animals directly in the tissue. As opposed to eight to twelve months, the time required to produce a knock-out mouse strain, only a few weeks are needed to conduct an experiment using this new technique. Another advantage is that several genes can be turned off simultaneously. This is a crucial issue in research, since many diseases are not based on a single switched-off gene, but rather on several ones.

In spite of this, RNAi also has its limitations. Knock-out mice will still be necessary in the future, since the new method does not allow switching off genes completely, but by 70 per cent at best. Nevertheless, many scientists see promising perspectives for RNAi, amongst other areas also for the treatment of diseases.

"These new systems are much more precise than conventional ones. The experimental results are not distorted ..."

Kurt Bürki, University of Zurich





Research with non-human primates is performed in industry and at universities. Due to their similarity to humans, research using primates can deliver unique data. For the same reason, such experiments also repeatedly give reason to debates.

Experiments using primates

Disputed experiments

“The long-tail macaques are trained to cooperate in the experiments. This reduces stress, both for the animals and the caretaker, and the results of the experiments are improved,” explains Walter Stamm, animal caretaker at Roche. Still, experiments using primates remain disputed.

Experiments with primates are disputed mainly on ethical grounds, for these animals are our closest relatives. The great apes especially – gorillas, orang-utans, and chimpanzees – should not be used for experiments. Some EU countries (Austria, Sweden, and the Netherlands) have already implemented this request. Animal welfarists demand that such a ban should already apply even if the animals are submitted to only minor or no distress. This opinion is also held by a majority of the members of the Swiss Federal Committee on Animal Testing (EKTV) and the Swiss Ethics Committee on Non-

Human Gene Technology (ECNH/EKAH). However, a majority of the members of the Committee for Science of the Swiss National Council is of the opinion that a rigorous harm-benefit analysis would serve people better than an explicit ban.

In the view of the EKTV and ECNH committees, research using other, non-human primates (such as rhesus monkeys and long-tail macaques) should only be approved after a thorough consideration of the ethical issues, a comprehensive harm-benefit assessment, and only with the “greatest restraint”.

A few research projects using primates causing severe distress are also being conducted in Switzerland. For certain questions, this is indispensable – for example, when it comes to the safety of pharmaceutical products. In Switzerland in 2005, 148 primates were used in biological and medical basic research and 260 in the areas of discovery, development, and quality control in medical sciences (transplantation medicine, asthma research, brain research, neurobiology, pharmacology). The research was conducted in industry and at universities.

Research using long-tail macaques: Trust and cooperation are vital

Walter Stamm is one of four animal caretakers at Roche that takes care of long-tail macaques. Each caretaker is responsible for approximately 15 animals. The macaques are used for pharmacokinetic studies, during which the distribution of substances throughout the body is evaluated. Since these tests cause the animals little distress, the same animals are kept for years and used again and again for testing.

“All of the macaques that we use in research come from controlled breeding stations abroad that breed the animals exclusively for use as laboratory animals. This largely ensures that the animals are free of infectious diseases and parasites. Nevertheless, we examine the animals’ health status thoroughly when they first arrive.

Before we begin with the training programme, in a first phase it is essential to build mutual trust between the human being and the animal. It is important for the animals to learn that I do not intend to do them harm. Trust can be built, for example, during feeding: The animals become accustomed to being fed by hand. By the way, primates do not all like to eat bananas. For some, it is only a preference acquired over time. When feeding the monkeys, it is my job as a caretaker to respect the rank order within the group. First I feed the highest-ranking male, followed by the second-ranking, or beta male, and then the rest of the group.

No training without trust

To build trust, it is also important to provide the animals with the time necessary to become accustomed to their new environment – to noises, the daily routine, the food, and, of course, to us care staff. Once trust has been established between the caretaker and the animal, the training programme can begin. The animals learn

to be handled directly by humans by being petted and stroked every day. They have to learn to accept my reaching into the cage. In the next phase, the primates learn to voluntarily present an arm or a leg from the cage. This is important for the experiments to come, when blood samples must be taken from the animals (see picture below left). In the tests using long-tail macaques, usually a substance is administered and then blood samples are taken over the course of two to three days. After that, the animals have a resting period that lasts a month. Thus, the animals are trained in order to cooperate during the experiments. This reduces the distress for the animal and also for the caretaker when collecting blood samples and the research data are more reliable. For this reason, a lot of time is invested in training the animals, usually one to one and a half years.”





Computer mouse, not mouse

“We do not replace animal testing, but we reduce animal testing,” says Angelo Vedani. Vedani focuses on computers to reduce the number of animal tests in biomedical research. There have been enormous advances in computational technologies in the last twenty years.

Angelo Vedani's dream is evident: One day, computer mice shall be the only mice required to conduct toxicological testing. Such tests serve to determine harmful effects of substances. Vedani specialises in computer aided drug discovery (CADD). CADD is now routinely being used in the pharmaceutical industry in order to design optimal substances on the screen and also to identify possible adverse effects of the substances. Angelo Vedani explains his CADD project in an interview.

What are the advantages of CADD?

Angelo Vedani: In the course of the development of new pharmacological substances the question might be which of 20 active substances to develop further. Our computer models can predict which substances to withdraw from further testing. Thus, we do not replace animal testing, but we sometimes prevent very distressful animal tests.

Like a lock and key

Many active ingredients in drugs unfold their effects by binding to receptors. A receptor is a protein that, for example, is located on the surface of a cell and transmits signals into the cell. The better an active ingredient binds to the receptor in question, the better the medication will work. The better an active ingredient binds to a receptor that triggers an undesired effect, the greater the probability that the active ingredient will cause side effects.

Which other advantages exist?

One advantage is that computational methods are reproducible – this means that scientists will obtain the same results on a test regardless of whether they are at work in London or Peking. That is not always the case when performing tests on live organisms. We can test an active substance within a short period of time. And computer methods are cost-saving in comparison to animal tests, because all that is needed is a powerful computer and the right software.

How exactly does the method work?

At the present time, our system contains models of eight important human receptors (see box) – amongst others the androgen receptor, estrogen receptor, and the enzyme cytochrome P450. We can model how strongly a candidate substance will bind to one or more of these receptors. If its binding affinity is strong, the candidate substance will be withdrawn from the evaluation pipeline, because it is very likely to cause undesired side effects.

What are the limitations of the model?

Those substances that fail in our tests are withdrawn. But this does not necessarily mean that the other substances will not cause side effects. It might very well be that a candidate substance

would bind to some other receptor that is not recorded in our database. It is also possible that the structure of the candidate substance will change in the living organism and will therefore bind to other structures. We are currently working on recognising such false negative predictions beforehand.

What remains to be done?

At present there is not yet one single in silico method (computer-based method) that has been accepted worldwide for toxicological testing. Together with the authorities we are currently working to establish the criteria that a software programme must fulfil in order to gain regulatory acceptance.

Which contribution can in silico methods make to REACH, the new EU testing programme for chemicals (see page 30)?

We can make an important contribution to this programme. Many environmental chemicals bind to exactly the same receptors that we already have in our system. We therefore have a good basis for predicting, which substances will cause harmful effects. However, the EU has not yet decided, which methods it will accept and use for REACH.

Replace
Reduce | Non-invasive methods
Refine

Markus Rudin
University of Zurich
and ETH Zurich



“Reductions by almost 80 per cent”

Non-invasive imaging techniques are methods to observe an organism (a mouse, for example) without having to penetrate the organism. Thus, these are gentle methods that have a number of advantages in regard to animal testing.

The research area of non-invasive imaging techniques encompasses a wide range of methods for observing organisms. Which are these methods?

Markus Rudin: They include, for example, methods such as the conventional X-ray technique or its further development, the computerised tomography (CT). Another important method is the so-called magnetic resonance imaging (MRI), which allows detailed structural and functional investigation of the brain, for example. Some other methods are positron emission tomography (PET) and fluorescence tomography.

What are the advantages of these methods?

Apart from the fact that one does not have to intrude into the body with any instruments, there are three other important advantages. In principle, the method is identical for humans and animals. This results in a better comparability of the results. Another advantage comes into effect when studying long-lasting chronic diseases, in particular: Using non-invasive methods, the researcher can observe the same animal

over a long period of time, without having to kill the animal for a specific investigation. One can recognise changes in the animal that are not visible externally and that might cause pain, which then points to the need to stop the test early. Fewer animals are therefore needed to perform an experiment, because each animal serves as its own control. At best, the savings can be 80 per cent and higher.

What is the third advantage?

The third advantage is that we can study an effect in a living organism. For example, we can give a substance to a mouse and then observe how the organism as a whole reacts to it.

To which extent are these methods being used in laboratories nowadays?

Imaging techniques are finding increasing use in animal testing. In spite of this, wide-spread use and acceptance of the techniques is being held back, because many of the newly developed methods are not yet, or not yet comprehensively, validated. Nevertheless, they show great potential, especially for the phar-

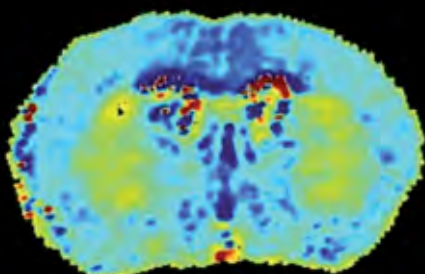
maceutical industry. And, by the way, the pharmaceutical industry has many years of experience with such methods. The industry is a pioneer in this area.

Are the methods being used at the universities?

Presently, this type of research is also increasingly being performed at the universities, such as at the University of Zurich and at the ETH Zurich, where the Zurich Center for Imaging Science and Technology (CIMST) was opened in 2005. On the one hand, this Center has the mission to promote the development of the methods and on the other hand to promote biomedical research at the University and the ETH Zurich.

Which are the limitations of the methods?

At the moment there are limitations as to resolution and sensitivity, with these two aspects being partly connected. For example, it is not yet possible to image individual cells or cell assemblies in a living organism with these techniques. For most of the techniques, however, such limitations are not of physical origin, but rather dictated by the current state of the technology. Therefore I do believe that we can expect to see a lot happening in the next couple of years.



Functional MR imaging

Neuroactive substances cause changes both in blood flow and blood volume in the respective area of the brain. In this computer image, areas with a high blood supply appear in red.

Implementation is decisive

Whether the methods will indeed be used and benefit the animals is decided in the world of practice. It can take years for a method to become officially accepted by the authorities, for it has to be studied in lengthy tests first.

Many experts agree: While the 3Rs as a concept may be a matter of course nowadays, implementing the 3Rs in routine laboratory practice takes constant effort. When it comes to implementation, two areas are to be distinguished (see figure below):

- **Legally required testing** (such as toxicological tests for chemicals, drugs, and vaccines).
- **Basic research** with, in principle, free choice of research methods. Scientists generally choose the experimental method that leads to the goal most reliably.

The Swiss Federal Animal Protection Act regulates both of these areas. According to this legislation, an experiment shall not be approved if its scientific goal can be met with a non-animal test method that is valid according to the current state of knowledge.

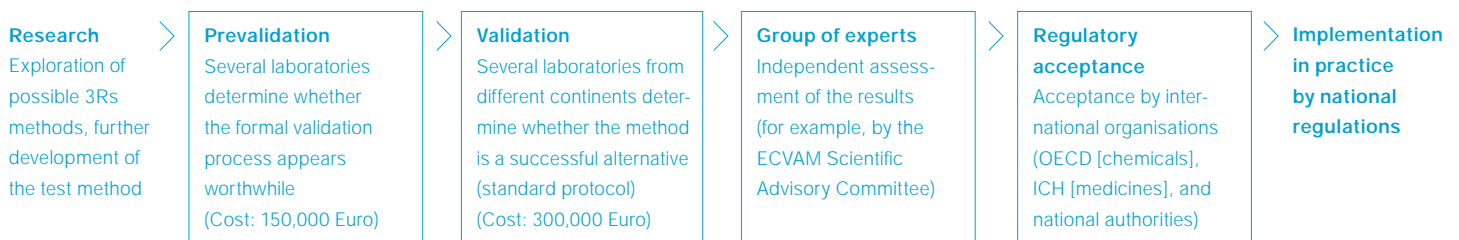
Legally required testing

As regards legally required testing, international committees determine, which 3Rs methods may be used. The protocols to perform tests with alternative methods have been laid down in detail. At first, these protocols were prevalidated

- that is, they were tested for their suitability. In Europe, ECVAM (European Centre for the Validation of Alternative Methods) is responsible for such scientific validation. By the spring of 2007, a total of 25 alternative methods have been validated by ECVAM, and ten of them have been accepted by regulatory authorities, such as the OECD (all of these are methods for the toxicological testing of chemicals or medications). At the same time, another 40 methods were in the final phase of the time-consuming and costly validation process and 190 in the initial phase.

The long road to implementation

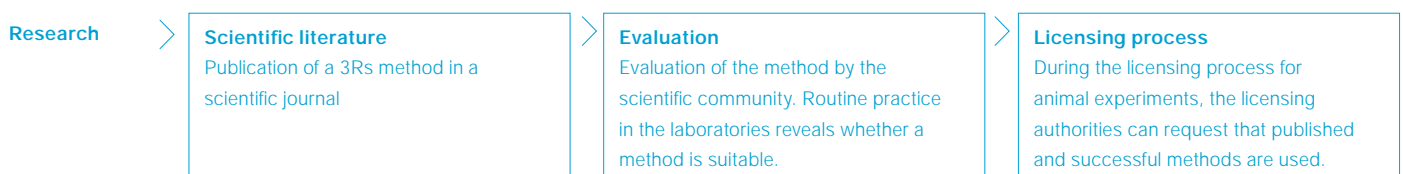
For legally required testing (example: toxicological tests): periods of 5 to 10 years



Validation is the process by which the scientific significance and reproducibility of a method are established according to defined criteria. During validation, the method undergoes practical testing in several, independent laboratories and ideally on different continents. A large number of different test substances are tested following a precisely defined procedure (standardised protocol). If the results obtained conform, the first hurdle has been

taken. Next, the significance of the method in comparison to the respective animal test must be determined. A successful validation is the necessary prerequisite for an official acceptance of the test method by the regulatory authorities. The authorities stipulate which tests must be conducted, for instance, for the registration of chemicals or the authorisation of medications.

In basic research: period of approximately 3 to 5 years





The long road to validation. In order for new 3Rs methods to become applicable for legally required testing, they first have to be validated. Validation ensures that the method is indeed an alternative that yields safety levels that are comparable to the animal test.

The freedom of basic research

All of the methods evaluated by ECVAM, however, are of little importance for basic research at universities or for applied research in industry. The reason for this is that in these areas neither validations nor choice of 3Rs methods are mandatory. Instead, freedom of research is essential. Methods are being disseminated by publishing them in scientific journals, so that every researcher can try them, test them, and improve them. Once a method is a part of the scientific literature, it gains official status and the authorities can refer to it.

In Switzerland, the implementation of published methods takes place via the official authorisation process. A scientist who submits an application for a license to conduct an animal experiment has to indicate whether other methods exist for the purpose he is intending to pursue that do not use animals or that cause less distress to the animals. If the researcher overlooks an available method, the licensing authority will call the researcher's attention to this and ask why the method was not taken into consideration. Whereas the authority can not force the re-

searcher to apply a specific method, the license application as it stands will probably not be approved.

—
<http://ecvam.jrc.cec.eu.int>

Knowledgeable staff makes for better animal testing

One of the greatest advances in animal testing was achieved in the area of training and continuing education. Today, everybody in Switzerland who works with laboratory animals completes a professional training course and is required to take continuing education courses regularly.



Since 1999, a Federal Ordinance has governed the specific training and continuing education requirements for individuals carrying out or supervising animal experiments. Apart from a basic course in biology and medicine, the training also conveys knowledge in the area of the 3Rs principle. "Better trained personnel lead to better animal experiments. This has probably been one of the biggest advances in the area of animal testing in the last ten years," says Peter Maier, scientific advisor to the 3R Research Foundation. Better education and training leads to a more responsible handling of the animals during the experiments.

In a mandatory, one-week training course, participants acquire basic knowledge on animal testing. There they do not only gain practical experience in correctly handling the laboratory animals, but they also learn about the advantages and limitations of in vitro methods.

Learning via the Internet

In 2004, a web-based learning programme called the 3R Training Course addressed at persons who perform or supervise animal experiments was developed on behalf of the 3R Research Foundation. The training course, which aims at deepening the knowledge on the 3Rs methods is available in German or English. The programme has been approved by the Association of Cantonal Veterinary Surgeons. It is updated on a regular basis and offers the option of taking a test on the course material via the Internet.

<http://3R-training.tierversuch.ch>

Nowadays, great emphasis is put on the training and continuing education of persons that work with laboratory animals.

**“The 3Rs
are a concept, not
an ideology.”**

Peter Maier, University of Zurich,
3R Research Foundation



In the past, discussions on the application of the 3Rs principle were often ideological. Today this discussion has become somewhat calmer. Personnel, which is well educated and informed, can decide whether animal tests are necessary for investigating a scientifically relevant question and, if so, which ones. Frequently, a combination of animal testing and in vitro methods delivers the best results. “The 3Rs are a concept, not an ideology,” explains Maier. Ideally, it is possible to gain sufficient information with in vitro methods to make animal testing superfluous.

During their training, researchers carrying out or supervising animal experiments also learn that neither the animal test nor an alternative method can offer 100 per

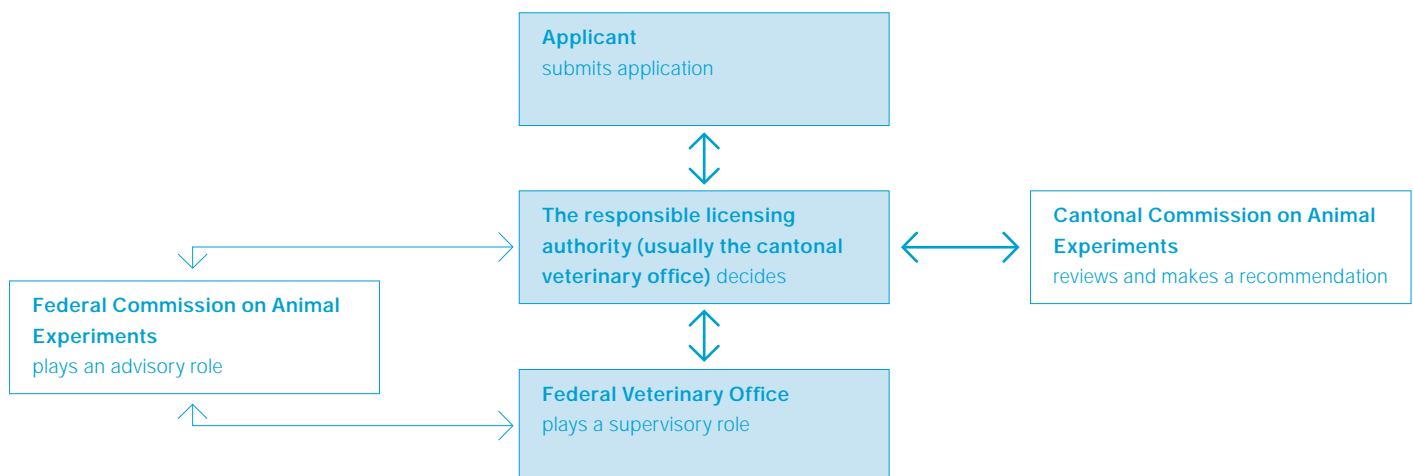
cent safety. It is not possible to re-examine all data, which was originally gained in rodents, in humans. For instance, it is self-evident that acute toxic dose tests can not be performed in humans. In such cases, the researchers can only perform a rough estimation. Therefore there are always incidences where substances that were previously tested and found to be safe in animals cause unforeseeable side effects in clinical trials with humans. One of the problems is that side effects in people, such as sweating, dizziness, nausea, or individual allergic reactions can hardly be revealed in an animal experiment.

The mandatory basic professional training course is followed by regular courses for continuing education. These are also go-

verned by the Federal Ordinance, and they are coordinated and monitored by the cantonal veterinary offices. Each year, all persons supervising and performing animal experiments must fulfil a day of continuing education in order to keep up with the state of knowledge. These persons include veterinarians, biologists, physicians, biochemists, biology laboratory technicians, and animal caretakers. They work at universities, at universities of applied sciences, in the pharmaceutical industry, or at institutes that are contracted to carry out animal testing.

www.bvet.admin.ch/themen/tierschutz

How does licensing to perform animal experimentation work?



The license application as a quality control

During training, great importance is attached to ensuring that the researchers learn to correctly describe and give the reasons for an animal experiment. Before each animal experiment, an extensive application for a license to perform the animal experiment must be submitted that states the aim for conducting the test and describes the methodology chosen. The researcher must also perform a harm-benefit analysis (harm to the animal versus anticipated knowledge gain for humans) and lay it open in the application form. Thus, the application for a license is a valuable instrument that requires research-

ers to examine their plans: What exactly do I want to investigate, and how? This ensures that the researcher weighs the procedures he is planning in every detail – including the question of how he will treat pain in the animals. The Cantonal Commission on Animal Experiments reviews the applications and makes recommendations. If there is nothing to object to, the license to perform the animal experiment is granted. This procedure assures and legitimates the quality and the indispensability of the animal experiment. Such a procedure, which depends on external reviewers, is unique in basic research and is not found in any other area of research.

Globalisation also regarding the 3Rs

The 3Rs concept is global. Many industrialised countries follow these principles when it comes to animal testing. In many European countries, the four interest groups – government authorities, animal protection, industry, and academia – have joined together to form 3R organisations.

Nevertheless, smaller and larger differences can be found from country to country in regard to the performance of animal experiments. These also encompass cultural differences. In most European countries and in developed countries on other continents, the expected degree of severity for the animals, classified using numbers or letters, is stated on the application for a license to perform an animal experiment. In Switzerland, at the end of each year, scientists are even required to retrospectively report to the Federal Veterinary Office the severity of the distress actually experienced by each individual animal. The United Kingdom, however, does not yet have an official system to classify

the severity of animal experiments. In the United States, the Animal Welfare Act does not apply to rodents – in Switzerland 90 per cent of all laboratory animals are rodents. Some countries, like Switzerland, are known for a consistent monitoring of the regulations as well as for inspections of the laboratories in the area of animal testing. Others, such as some developing countries, are more lax in applying their standards or have none at all.

A platform for exchanging ideas

In many European countries, national consensus platforms have been formed, in which the four interest groups – industry, government, academia, and animal

protection – work together towards the 3Rs. The European umbrella organisation of these 3R organisations is called ecopa (European Consensus Platform for Alternatives), and it is based in Belgium. ecopa is a platform facilitating the mutual exchange of scientific information and expertise to promote the 3Rs. It was founded in the fall of 2002 with the active support of the 3R Research Foundation. The aim of ecopa is, amongst other issues, to coordinate very large and complex projects in the area of 3R research. ecopa has also established contact links to similar organisations in Japan and in the United States.

REACH chemicals testing programme – a special case

The EU is planning to test 30,000 chemicals for harmful health effects and environmental safety in the coming years. For a majority of the chemicals that were put on the market prior to 1981 this basic safety information is lacking. These chemicals therefore pose a certain risk for people and the environment. Under REACH (Registration, Evaluation and Authorisation of Chemicals), all substances manufactured or imported in the European Union in volumes greater than one tonne per year will have to be tested and then registered in a central database managed by a new European Chemicals Agency. Chemicals that raise particular concern, because they are carcinogenic, for example, will additionally require an authorisation for further use.

The REACH programme will cost between four and eight billion Euro and will begin in the year 2008. While this is good news for consumers, REACH will have serious consequences for laboratory animals. REACH will cause the numbers of animals used for toxicological testing to increase considerably in the next ten

years. Estimates of 60 million animals were initially made. Today, it is predicted that three to four million research animals will be required at the minimum. For this reason REACH specifically states that animal testing for the purposes of this regulation shall be undertaken only as a last resort and that research for developing and validating alternative methods to refine, reduce, or replace animal testing will be promoted. As the actual animal testing does not begin until 2011, there is still some time to develop and test appropriate 3Rs methods. The EU is investing 80 million Euro for research projects in this area.

Such methods will then be validated by ECVAM. Thomas Hartung, Head of ECVAM, estimates that the alternative methods will result in a 50 per cent reduction in the number of laboratory animals used for the REACH programme. A further 20 per cent reduction will be achieved through the use of computer methods.

http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm

Spreading the 3Rs concept in Europe

In mid 2007, 15 countries were participating in ecopa: Austria, Belgium, the Czech Republic, Denmark, Finland, Germany, Hungary, Italy, the Netherlands, Norway, Poland, Sweden, Spain, Switzerland, and the United Kingdom (see map).

A further organisation is ECVAM, the European Centre for the Validation of Alternative Methods (see also box on page 30). ECVAM aims at increasing acceptance of non-animal research and, on the European level, to promote the independent validation of adequate alternative methods. ECVAM also maintains a database, which encompasses detailed information on methods that have already been validated.

The American counterpart of ECVAM is ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). The organisation was established in 1994. Its purposes are very similar to those of ECVAM.

www.ecopa.eu



ecopa is the umbrella organisation of all national 3R platforms.

- Full members of ecopa
- Associate members of ecopa



ECVAM is located in Ispra, Italy. ECVAM is an institution of the European Commission.

Thomas Hartung,
University of Constance/ECVAM

A vision brings about success

Since 1987, the 3R Research Foundation has funded research projects with the aim to develop non-animal test methods. To date in mid-2007, the Foundation has supported a total of 108 research projects.

At the particular initiative of Susi Eppenberger and Hugo Wick, members of the Swiss National Council, the Swiss Parliamentary Group on Animal Testing was formed in the fall of 1985. This came about in response to many years of debate on the petition for a referendum "to ban vivisection", which was turned down by the voters during the vote of 1 December 1985, and to the next attempt to petition "a ban on animal testing and vivisection", which was not accomplished. The Parliamentary Working Group looked for a way to bring together the interested parties – politicians, government, animal protection, and industry – to find objective solutions to the problems. "We wanted to do something for laboratory animals, without driving the pharmaceutical industry out of Switzerland," says Hugo Wick, former (CVP party) member of the National Council and chairman of the 3R Research Foundation from 1995 to 2007.

And so the idea of a "Financial Pool 3R" assumed shape. It was its goal to promote research in the area of the 3Rs methods with funds from the Federal Government and those pharmaceutical companies involved in research, as represented by Interpharma.

Funds have been flowing for 20 years

On 13 February 1987 the step was accomplished: the 3R Research Foundation was launched by three members of Parliament, Interpharma, and the Fund for Animal-Free Research (FFVFF). The same year, the first research grants were awarded.

The 3R Research Foundation supports a broad range of research projects, provided that they present promising new approaches to replacing, reducing, or refining animal testing. Currently, it is especially important to apply the 3Rs principle in the

areas of biomedical research and drug development. That is where the greatest number of animals are used, and for that reason, scientists are needed that, while focusing on their research questions, are also willing to develop new 3Rs methods in the course of their research projects. "This is precisely where the Foundation can support the scientists and can encourage new ideas," explains Peter Maier, scientific advisor to the 3R Research Foundation. Frequently, grants are awarded by the 3R Research Foundation as a complement to other research grants, such as the Swiss National Science Foundation.

The Foundation attaches great importance to the publication of research results upon completion of the projects. This is the only way to ensure that the insights gained come to the attention of other relevant researchers and will be taken into consideration in the future.

Milestones

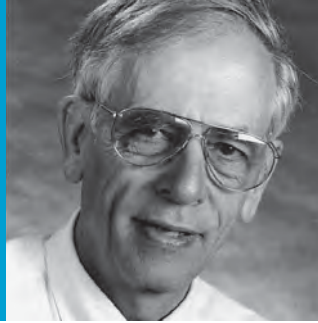
■ Since its founding in 1987, the 3R Research Foundation has approved 108 projects for funding and awarded a total of approximately 15 million Swiss Francs in grants (to date, mid-2007). A list of all projects with English descriptions of the project goals, methods, and results is available on the Foundation's website. Each year, the Foundation approves on average five projects, which then mostly receive funding for a period of two to three years.

■ Since 1994, successful research projects have been highlighted in a short, illustrated publication called the 3R Info Bulletin. To date in mid-2007, 36 issues of the Bulletin have been published. The 3R Info Bulletin is sent to more than 1,000 interested readers and is also available on the Foundation's website.

■ In 1995, in 29 different laboratories in Switzerland, the in vitro production of mouse monoclonal antibodies using bioreactors instead of mice was validated, achieving a breakthrough for this in vitro (test-tube) method.

■ Available since 2005, the Foundation offers an online training course for the personal and professional training and continuing education of persons that carry out or supervise animal experiments. Using the texts, illustrations, links, and documents provided, interested people can learn about 3R topics and the legal regulations governing animal testing.

Hugo Wick is a founding member of the 3R Research Foundation and served as Chairman of the Foundation for 12 years.



The Administrative Board unites different interests

As laid down in the Foundation statutes, the nine-member Administrative Board is made up of two members representing the industry, two representing animal protection, two from the Federal Veterinary Office, and three from the Parliamentary Group for Animal Experimentation Issues.

The Board is responsible for the management of the 3R Research Foundation. In this task, the Board is supported by the Scientific Advisor and the Secretariat. The Board approves or rejects research grant applications according to its guidelines and to defined priorities and upon the recommendations of the Evaluation Committee.

The Evaluation Committee ensures sound expert review

The Evaluation Committee consists of at least four (presently nine) scientists from different specialist fields. They are experts from academia, industry, administration, and animal protection. As a body made up of specialists in their respective specific research areas, the Evaluation Committee as a whole provides a competent and balanced peer review of the research grant applications. The scientific advisor of the Foundation is the Chair of the Evaluation Committee.

The Evaluation Committee reviews the applications for funding of a research project and makes recommendations to the Administrative Board as to approval or rejection of the application and the size of the grants to be awarded.



Hans Wyss
Federal Veterinary
Office



Susanne Scheiwiller
Animal-Free Research



Authority

Promoting 3Rs methods: An official mandate

The Federal Veterinary Office (FVO) has been charged to promote the recognition and the implementation of 3Rs methods. Therefore the promotion of these methods is also one of the priorities of the FVO's research. This is largely assured through the 3R Research Foundation. The FVO is actively involved in the Foundation through its annual financial contributions and through participation in the Evaluation Committee and representation on the Administrative Board.

But how does the work of the Foundation benefit animal welfare? The projects funded by the Foundation are strongly oriented towards basic research. The results generated are pieces in a mosaic that lead to selective improvements in research and later on also indirectly influence the Ordinances on animal protection or their executive provisions. Here are two examples for this: Based on research made possible by the Foundation, the FVO issued Guidelines on the production of monoclonal antibodies. Now these antibodies are produced almost exclusively without using animals in Switzerland. As the result of another 3Rs project funded by the Foundation, infections caused by certain bacterial toxins (the Clostridium toxins) can be detected by PCR analysis. In the past, it was only possible to test for these toxins using animals.

Animal protection

Refinement is not enough

In the mid-1970s, the 3Rs concept led to a shift in the relationship between animal protection and science, by enabling a dialogue between the scientists and representatives from animal protection organisations. Until today, the 3Rs principle has found its way into a number of legislative texts, so that now, almost 50 years after propagation of the 3Rs by Russell and Burch, it is time to ask to which extent the 3Rs are actually being applied in research laboratories in Switzerland. As regards Refinement, there is good news to report. Improved housing and care of animals in enriched environments is now the case in many laboratories. The use of analgesics and the discontinuation of testing if the animals are suffering too much are hardly questioned any longer by Swiss scientists. But how far along are we in regard to Reduction or to the Replacement of animal tests? In these areas, a lot remains to be done. Especially in basic research, animal testing is still the method of choice. It is in this area, in particular, that the willingness to switch to alternative options is rather low – most likely also because the researchers fear that important pieces of information will be missed when using such methods and because an in vitro test is likely to receive less recognition in scientific circles. Studying complex processes in intact animals continues to enjoy high acceptance. Furthermore, scientists conducting animal research are oftentimes not familiar with in vitro methods.

After almost 50 years since the introduction of the 3Rs principle it is time to award replacement methods the status and importance that they deserve – and this not only for animal welfare reasons but also on scientific grounds. Already in 1959, in their standard work Russell and Burch insisted that it does not suffice to stand up for Refinement “only”. The goal must always be the Replacement of animal experiments.

Thomas Cueni
Interpharma



Max Gassmann
University of Zurich



Industry

Dialogue, not confrontation

The discussion on animal testing is shaped by the conflicting priorities of using and protecting. The founding of the 3R Research Foundation 20 years ago was a pioneer achievement in Europe. The joint efforts of politicians, animal protection, authorities, and the pharmaceutical industry brought different interests to the table for a common goal: animal protection through promotion of research in the area of the 3Rs. This research was to deliver insights resulting in better scientific findings as well as in less distress for the laboratory animals.

Interpharma's support of the Foundation is a sign of the industry's clear commitment that animal experiments should only be conducted insofar as they are indispensable for scientific knowledge gain. Accordingly, distressful animal tests must be restricted to the indispensable extent. However, despite the progress achieved, it must be noted that for many diseases there is still no cure, and the safety and efficacy testing of drugs still requires the use of animals – for the patients' protection. The work of the 3R Research Foundation is not yet accomplished. Today and in future, it will proceed in the spirit of not confrontation but dialogue and cooperation.

Research

It is the goal that counts in basic research

Animal testing is necessary in numerous areas of biomedical research and at many university institutes. The researchers involved are natural scientists, especially biologists, veterinarians, and medical doctors, as well as biology lab technicians, doctoral candidates, and department assistants.

It is intrinsic to research that the research question, or the goal of the project, takes top priority. The scientist has the responsibility to use the method that is appropriate to reach the research goal in an optimum way, for the scientist also bears the consequences of success or failure. The researcher must decide whether the animal experiment is necessary or whether the goal can be reached using other means.

The 3Rs principle provides the best framework for making that decision. The 3Rs are prerequisites for conducting good research and for obtaining reproducible results. Therefore it is especially at the universities that there is a constant need for qualified training and further education in the area of laboratory animal science in order to achieve improvements in experimental animal testing and to update the existing knowledge on possible 3Rs methods. The universities respond to this challenge as a matter of high priority.

The 3R Research Foundation

The 3Rs stand for Replacement, Reduction, and Refinement. This implies the concept to replace animal testing, to reduce the number of animals used in testing, or to refine methods to minimise the distress for research animals. The 3R Research Foundation provides funding to research projects in the area of the 3Rs. It is a joint undertaking by the Parliamentary Group for Animal Experimentation Issues (Government), Interpharma (Swiss Association of Pharmaceutical Companies that perform research), and the Fund for Animal-Free Research (formerly FFVFF). Financially, the 3R Research Foundation is supported equally by the pharmaceutical industry association (Interpharma) and the Swiss Federal Veterinary Office.

The Administrative Board of the Foundation has appointed an Evaluation Committee, which has been assigned with the task to review the proposed research projects and applications for grants. The members of the Evaluation Committee are renowned scientists from academia and industry as well as representatives of the Government and of animal protection.

The projects that were funded by the 3R Research Foundation have made a contribution to developing new and better methods and thereby to reducing the number of animals used in experimentation in Switzerland. Since it was established in 1987, the Foundation has funded over 100 research projects.

