

# 2017

**ANNUAL REPORT**  
THE DANISH 3R-CENTER







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PHOTO: CHRISTINA HAUSCHILDT

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## CHAIRWOMAN'S FOREWORD

### DEAR ALL

The Danish 3R-Center covered a wide scope of activity to promote its 3R efforts during 2017. We granted research funding and issued newsletters; we held our annual international symposium where we also presented the 2017 3R Award; we took part in many different conferences and meetings where we discussed and presented our viewpoints; and we also focused on disseminating information on our website – to name just a few of our tasks.

In addition, we held the event “*Videnskab med øl til*” (Pint of Science) and took part in “*Science Calling*” because we take our dissemination obligations seriously – not only in the world of

laboratory animals and alternatives, but also in the public in general, including schoolchildren.

In 2017, we also launched a project aimed at studying the possibility of reducing the number of laboratory animals which experience severe suffering during procedures in Denmark. Further details about this project are found in this Annual Report.

We also launched the project “*Possibilities and barriers to replacement in animal experimentation*”, which is presented below.

In November, the Danish 3R-Center hosted yet another international symposium. The many attendees and numerous presentations demonstrated that there is still an enormous potential for collaboration to create progress within the 3Rs.

At the symposium, the results of research projects supported in previous years were presented and it was also possible to see posters from several of the ongoing projects. This year, the 3R-Center has also supported three more new 3R research projects, which you can obviously read about in this report as well.

This year, veterinarian Grete Østergaard of the University of Copenhagen was the recipient of the 2017 3R Award. Grete and her colleagues have been working to ensure that the animal experimentation required for research and teaching at the University of Copenhagen is conducted in a manner that is as ideal as possible from a 3R perspective. Grete's enthusiasm for the cause was crystal clear in her acceptance speech at the 2017 symposium.



GRETE ØSTERGAARD, PHOTO: KIM GRANLI

The efforts of the Danish 3R-Center are also focused on the next generation. We have previously commissioned teaching materials about laboratory animals and the 3Rs for use at both upper secondary and lower secondary levels. For this reason, we worked to disseminate knowledge of this in 2017, an effort that is continuing into 2018 where we will present the material at the Danish Learning Festival.

I would like to take this opportunity to thank my fellow board members and the secretariat for their great dedication in 2017. It has been a pleasure working with you.

Last, but not least, I wish to express my gratitude to the Danish Animal Welfare Society, LEO Pharma, Lundbeck, Novo Nordisk and the Ministry of Environment and Food for their support of the Danish 3R-Center in 2017.

In 2017, a political decision was made to continue the Danish 3R-Center with support from the above-mentioned stakeholders – which will now also include DOSO (Cooperation Body for Danish Animal Welfare Organizations), which decided to support the centre, and naturally we are very pleased with this.

With the decision to continue the centre, we can continue our efforts to create a leading environment for the dissemination of information and application of the 3Rs to benefit both research and the laboratory animals.

**Christine Nellemann**

Chairwoman of the board  
of the Danish 3R-Center

## THE BOARD OF THE DANISH 3R-CENTER WILL CONTINUE

***Press Release,** the Danish Veterinary and Food Administration, 9 November 2017*

All board members will continue working to improve the conditions for laboratory animals in Denmark.

All members of the board of the Danish 3R-Center have just expressed their willingness to continue their board work at the centre for four more years. In so doing, the seven experts will continue participating in efforts to improve the conditions for laboratory animals in Denmark.

Christine Nellemann, Director of the National Food Institute, is the chairwoman of the board. Like the other six members, she was

appointed by Esben Lunde Larsen, Minister for Food. In recent years, the centre has supported a wide range of research projects aimed at protecting and limiting the use of laboratory animals.

The centre has hosted several international conferences on animal experimentation and has also commissioned the preparation of teaching material for both upper secondary and lower secondary schools. In the years ahead, the centre will also focus on supporting research projects and disseminating knowledge to improve conditions for laboratory animals in Denmark.

## PROLOGUE

# THE EU AND ANIMAL EXPERIMENTATION

A new, updated European Directive on the use of laboratory animals for scientific purposes came into effect on 1 January 2013 and the directive has now been implemented throughout the European Union. As a result, common regulations now govern how laboratory animals must be accommodated and cared for, and the regulations also stipulate common requirements for obtaining permission to use laboratory animals.

Denmark had already implemented quite detailed requirements and thoroughly prepared procedures in this area. The requirements being introduced had already been used for accommodation and care in Denmark for several years.

The biggest new features were that the individual member states had to create animal welfare bodies at all animal experimentation facilities and set up a so-called national committee. The requirements for annual statistical reports were also made more stringent. The requirement for ongoing registration of the severity assessment of each individual animal was especially a new way of working.

Although at the start of 2017, it was only four years into applying an almost brand-new set of regulations at EU level, the European Commission was – pursuant to the directive – under an obligation to analyse the directive and determine whether it was working as intended. In addition, the directive stipulates a requirement that the European Commission must look into whether the plan to phase out the use of non-human primates caught in the wild can be complied with.

The analysis was conducted by sending questionnaires to Member States' authorities, researchers, breeders and suppliers, as well as to stakeholders in the area of animal welfare, such as animal welfare societies, 3R organizations and others. In addition, there was a questionnaire for organizations particularly interested in alternatives relating to education and teaching.

In Denmark, the Danish Veterinary and Food Administration (regulatory agency of the area) and many different researchers and animal welfare bodies, as well as the Danish 3R-Center, responded to the survey questions. Also, Danish NGOs could respond to survey questions either directly or via their international cooperation.

Finally, the European Commission also brought in expert knowledge from the *EU Reference Laboratory for Alternatives to Animal Testing* (EURL ECVAM), the *Scientific Committee on Health, Environmental and Emerging Risks* (SCHEER) and the Commission's response to the citizen initiative *Stop Vivisection*.

In the report, published on 10 November 2017, the European Commission concluded that the deployment of the directive is well under way. The establishment of animal welfare bodies in particular has been successful, and the Commission also found that improved standards had been implemented for research procedures, that there was keener focus on the 3Rs and the Culture of Care concept and higher transparency as well as an increasingly greater appreciation of the correlation between good animal welfare and good research results.



The analysis also concludes that there is still much work to be done in individual Member States to achieve uniform assessments of applications to use laboratory animals, just as access to statistical information about laboratory animals – and the quality of this information – could be improved in many instances.

Although this may be true of the situation in some countries, we have made great progress in Denmark. With only one overarching body (Animal Experimentation Council/Animal Experiments Inspectorate) to assess all applications to use laboratory animals in Denmark, we have a highly uniform assessment process, and the Animal Experiments Inspectorate ensures the quality of the annual statistical information on an ongoing basis.

### Non-human primates

The analysis of the use of non-human primates was conducted by a task force of experts (in which Denmark also participated) and through surveys of the conditions at breeders and suppliers of European and non-European origins. The overarching conclusion is that the Commission upholds the requirement that non-human primates caught in the wild may no longer be used for experiments in Europe five years after the report's publication (10 November 2017).

### Grassroots initiative *Stop Vivisection*

In 2015, the grassroots initiative *Stop Vivisection* submitted a petition with 1.7 million signatures to the European Commission with two demands: that the European Directive on laboratory animals be abolished; and that a prohibition against all animal experimentation be implemented within the EU by no later than 2020.

The Commission responded by stating that it shared the initiative's conviction that animal experimentation should be phased out, which is the ultimate objective of the European legislation. It was also assessed that even if there are obvious differences in how animal and humans react to different treatments, the use of laboratory animals has played an essential scientific role in the development of almost all the efficacious and currently existing reliable medical treatments and methods for protecting both animals and humans against illness and that animal experimentation has contributed results for the benefit of the environment. It was therefore concluded that at present it would not be prudent to implement a ban against the use of laboratory animals.

Four initiatives were launched, all of which aim to boost the development of alternatives to animal experimentation. As a result, the European Commission will work to achieve the following goals:

- Strengthen progress within the 3Rs, through knowledge-sharing by analysing technologies, information platforms and networks from all relevant areas where there is potential for boosting the 3Rs.
- In close cooperation with the Member States and international organizations, continue to support development, validation and implementation of alternative methods, both in relation to regulatory testing and in research.
- Actively monitor both the implementation of and compliance with the directive in all Member States and investigate whether all relevant legislation requiring the use of laboratory animals is sufficiently designed to replace animals with alternatives as quickly as possible when these exist.
- Hold a conference focused on using scientific advances to develop efficacious animal-free models and strategies for the purpose of phasing out the use of laboratory animals.

Reports on and results of all of the above initiatives can be found on the European Commission's website [http://ec.europa.eu/environment/chemicals/lab\\_animals/index\\_en.htm](http://ec.europa.eu/environment/chemicals/lab_animals/index_en.htm)



# 1

# RESEARCH PROJECTS

An important part of the Danish 3R-Center's work is to support 3R research projects as part of the annual granting of research funding. For this purpose, the Danish 3R-Center launches its own projects which can provide the centre with knowledge that can ultimately benefit laboratory animals. Last year's survey of familiarity with the 3R principles among users of laboratory animals and the two ongoing projects about "*Focus on animals exposed to severe suffering*" and "*Possibilities and barriers to replacement in animal experimentation research*" are good examples of this

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## RESEARCH GRANTS

The Danish 3R-Center manages the annual distribution of DKK 1.5 million to fund research within one or more of the 3Rs.

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### PRIORITY IS GIVEN TO PROJECTS':

- Quality
- Implementability
- Relevance

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### WHO IS ELIGIBLE TO APPLY FOR FUNDING?

Researchers affiliated with an organization, institution or company in Denmark.

It is possible to apply for up to DKK 500,000. The grants typically amount to DKK 100,000–500,000.

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Please note: It is possible to sign up for the Danish 3R-Center's newsletter at the centre's website ([3rcenter.dk](http://3rcenter.dk)) so you do not miss research-funding deadlines.

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# RESEARCH PROJECTS SUPPORTED IN **2017**

In 2017, the Danish 3R-Center received 34 applications for support, of which the following three received a total of DKK 1.3 million.

PHOTO: THE DANISH 3R-CENTER



# THE USE OF **PRIMARY KIDNEY ISOLATES** FROM HUMANS FOR STUDYING THE MOLECULAR ASPECTS OF BLOOD-PRESSURE REGULATION

**Henrik Dimke**, *University of Southern Denmark*

Hypertension (high blood pressure) is a major health problem. The Danish Heart Association estimates that roughly 950,000 Danes suffer from hypertension, an affliction which affects quality of life and increases the mortality rate.

The kidneys play an important part in a number of physiological processes and are particularly important for maintaining blood pressure. This is due to the kidney's ability to adjust the amount of salt excreted in the urine, which is done by regulating specific transport proteins. The changes to the kidney's salt transport are disturbed to varying degrees in most conditions of hypertension.

Drugs which increase the excretion of salt are the initial treatment of hypertension. Understanding how human kidneys regulate blood pressure is important for being able to understand the cause of this common affliction.

The purpose of the project is to develop protocols to use isolated tissue from a human kidney for studying hypertension and the molecular aspects which help maintain blood pressure. Tissue will be isolated from kidneys which, pursuant to an informed consent, are donated by patients hospitalized for kidney removal, usually due to kidney cancer. The use of these isolates will significantly reduce the number of animals required for such studies, as we can replace mice experiments with those using human kidneys. The tissue used would otherwise just be destroyed after removing the kidney.

In addition to reducing the number of animals, the use of human tissue in this type of research will also be able to overcome some of the apparent species differences naturally existing between mice and humans. A better understanding of how the human kidney works could heighten the understanding of the mechanisms which lead to hypertension and could help in the treatment of this disease.



# DEVELOPMENT OF **CELL-BASED ASSAYS** FOR ANALYSING THE ANTIBODY- MEDIATED PROTECTION AGAINST THE CHLAMYDIA BACTERIUM

**Jes Dietrich**, Statens Serum Institut

More than 100 million people become infected with the bacteria *Chlamydia trachomatis* every year. Women who become infected with the bacteria can develop pelvic inflammatory disease, which can eventually cause involuntary infertility. Antibodies that can neutralize the bacteria are essential in defending against it, and it is important that a vaccine against chlamydia is capable of generating these antibodies.

Vaccines against chlamydia are tested by using laboratory animals, but these animal models are far from ideal and require many animals per group to provide statistical significance.

Therefore, this project aims to develop new *in-vitro* methods based on cell lines instead of animals for testing the protective effect of vaccine-induced antibodies.

Specifically, the project involves the development of two *in vitro* methods which can measure 1) antibodies' ability to neutralize the bacteria; and 2) antibodies' ability to mediate that the bacteria is absorbed and destroyed by the immune system. It is hoped that these methods will reduce the number of laboratory animals used in chlamydia-vaccine animal experimentation and eventually comprise a new way of quickly testing a large number of vaccine candidates.



PHOTO: THE DANISH 3R-CENTER

# IMPLEMENTATION OF **PAIN THERAPY METHODS** FOR RATS BEING USED AS A MODEL FOR INFLAMMATORY ARTHRITIC PAIN

**Klas Abelson**, *University of Copenhagen*

Many people are afflicted with various types of chronic pain due to inflammatory illnesses or damage to the nervous system. At present, there are far too few available treatments against these pains, which cause severe suffering in the patients. At present, the use of laboratory animals is crucial for being able to develop efficacious drugs for treating chronic pain.

The use of laboratory animals in pain research is an ethical problem, however, because varying degrees of pain are often inflicted on the animals and occasionally the pain can be prolonged. Animals are rarely treated with analgesics, as these are suspected of adversely affecting the development of the experiment parameters.

In previous studies, the research group has shown how it is possible to treat a specific animal model for arthritis with the analgesic buprenorphine, improving animal welfare

without affecting the relevant experiment parameters. This explains why the model can still be used.

This research project will continue the above-mentioned studies. The first step will be to refine and optimize the current rat model by increasing the success rate in the development of the model and minimizing adverse effects such as leakage from joints to surrounding tissue, which is not relevant to the model.

Once an ideal method has been established, the next step will be to implement the most relevant pain-therapy method from previous studies to find a strategy for adapting the pain treatment in the current model with no or minimal effect on the experiment parameters. It will also be possible to use this knowledge to establish strategies and recommendations in other pain models

# OTHER PROJECTS

As previously mentioned, the Danish 3R-Center also launches projects on its own initiative. All current projects were launched in 2017 and they are presented below.

## FOCUS ON ANIMALS SUBJECTED TO SEVERE SUFFERING

As part of permission and reporting, animal experimentation in the EU must be categorized in terms of the severity of distress that the animals are expected to experience and actually do experience (Table 1).

The Danish Animal Experiments Inspectorate initially grants permission for maximum severity in the experiment procedures and subsequently, the researcher must file a report for each individual animal about what it actually experiences. Out of the 244,411 animals reported in 2015, it was reported that 2,168 animals (0.9%) had experienced severe suffering, which is the highest permissible severity classification.

In 2017, the Danish 3R-Center decided to visit the research groups where animals are reported as being subjected to procedures with the highest severity classification, with the exception of those that solely report on animals which die of natural causes. The

purpose of this was for the Danish 3R-Center to get an overview of this type of procedure in Denmark and to have an opportunity to identify possible development projects where the severity classification could be reduced. The purpose was not to discuss whether it was necessary to go up to the highest severity classification, as the permission for each experiment is only granted after thorough processing by the Animal Experimentation Council which considers, among other things, the severity of distress of the animals in relation to the beneficial effect of the procedures.

Therefore, Danish 3R-Center asked the Animal Experiments Inspectorate to list these research groups, adjusted for spontaneously deceased animals, and then contact the remaining groups to arrange a visit. Eight out of the twelve groups contacted so far have expressed interest in a meeting, and five of these have already been visited by the Danish 3R-Center in 2017.

One out of the eight groups turned out to be irrelevant in relation to the study, as an erroneous report had been filed. The remaining two will be visited in 2018, and an attempt will be made to establish dialogue with the remaining four research groups. Most of the researcher groups visited are based in public institutions.

The visits to the research groups are made by representatives of the board of the Danish 3R-Center. At the meetings, the researchers reviewed how the procedures are conducted and the specific purpose which the procedures are supposed to serve. It was obvious for each experiment that a highly limited number of animals have been involved out of a large group of animals used by the research groups, and that the purpose of the experiments was to serve compelling societal and/or patient-relevant needs.

Prior to these severe animal experiments, experiments of less severity had been conducted so that the procedures of highest severity were not conducted until it was absolutely necessary to establish documentation. Thus, in their board's experience, the research groups were keenly aware of the situation relating to the severely distressed animals.

A positive result of this was that a number of researchers gradually reduced the animals' severity of distress over the years. This has taken place at the same time that the Animal Experimentation Council has tightened the requirements for accepting a lower classification and reduced the maximum permissible severity, usually due the fact that animal experimentation models have generally become less severe. This has not manifested itself by changed levels of severity expressed in the reports, however. For example, death as an end point is only tolerated to a limited extent in the current European directive, and many of the animals that would previously have been kept in a procedure until death are now killed before this, e.g. when they have lost a specified amount of weight, where a weight loss of more than 20% is classified

as severe and more than 25% is deemed unacceptable in any circumstances.

There has also been a tendency to over-report animals in the most severe category, however. It is often possible that a substantial number of the animals admittedly authorized for inclusion in procedures of highest severity are removed from the procedure before reaching this level of severe distress. Even so, some researchers reported all animals as being in the highest severity classification and did not consider that only a small percentage of the animals were actually subjected to this level of severity. Therefore, even the estimated figure of 500–600 animals a year is possibly too high.

In some of the procedures discussed, it has been difficult to identify alternatives to existing procedures, whereas for other procedures it was possible to see modified end points which could possibly be used for a development and validation procedure. Discussions were also held on the possibility of pre-identifying animals to be subjected to the induction of disease or similar without subsequently responding to the treatment in the same manner as the majority of the animals and therefore do not end up contributing to the overall result.

These animals constitute a small share of the total number of animals used in experiments, but they are critical in terms of animal welfare because they are subjected to procedures that are more severe than those which animals normally would have been deliberately subjected to. Therefore, it is the hope of the Danish 3R-Center that in the years ahead it will be possible to reduce the need of individual research groups to conduct animal experimentation that must be reported in this category.

TABLE 1

Classification of animal testing by severity of pain, suffering, distress or lasting harm which the individual animal is expected to experience during the procedure, pursuant to European Directive 2010/63/EU.

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NON-RECOVERY

Procedures which are performed entirely under general anaesthesia from which the animal will not recover are classified as “non-recovery”.

MILD

Procedures on animals as a result of which the animals are likely to experience short-term mild pain, suffering or distress, as well as procedures with no significant impairment of the well-being or general condition of the animals are classified as “mild”.

MODERATE

Procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress as well as procedures that are likely to cause moderate impairment of the well-being or general condition of the animals are classified as “moderate”.

SEVERE

Procedures on animals as a result of which the animals are likely to experience short-term severe pain, suffering or distress or long-lasting moderate pain, suffering or distress and procedures that are likely to cause severe impairment of the well-being or general condition of the animals are classified as “severe”.



# POSSIBILITIES FOR AND BARRIERS TO **REPLACEMENT** IN ANIMAL EXPERIMENTATION RESEARCH

*Prepared in a cooperative effort involving **the Department of Food and Resource Economics (University of Copenhagen)** and **the Danish 3R-Center***

In 2016, the Danish 3R-Center initiated a study of Danish animal experimentation scientists' perception of and lessons learned from the implementation of the 3Rs (the study is presented in the section on the Danish 3R-Center's symposium).

The study identified a unique challenge in terms of *Replacement*. Although animal experimentation scientists have the best factual understanding of "*Replacement*" out of all the 3Rs, it is the 3R concept of which they have the poorest experience.

Therefore, in 2017, in cooperation with the University of Copenhagen, the Danish 3R-Center decided to conduct the study "*Possibilities of and barriers to replacement in animal experimentation research*".

As it is generally assessed that the most significant barriers to dissemination and implementation of "*Replacement*" are found in the environments at private-sector undertakings and public institutions which use laboratory animals, the project focuses on these environments. Thus, the purpose of the project is to study the scientific, social, cultural and attitudinal possibilities of and barriers to "*Replacement*" in environments using laboratory animals.

At the conclusion of the study in the spring of 2018, the results will be presented at various national and international conferences, as well as on the respective websites of the Danish 3R-Center and the University of Copenhagen, and in the 2018 Annual Report of the Danish 3R-Center.



PHOTO: KIM GRANLI

## EQUINE PALPATION /COLIC SIMULATOR – THE ARTIFICIAL HORSE

In 2015, representatives from the University of Copenhagen drew the attention of the Danish 3R-Center to the existence of a so-called *Equine Palpation/Colic Simulator* – an artificial horse, so to speak. The horse can be used by veterinary students in a wide variety of training exercises, sparing horses for these occasionally quite unpleasant and frequent sessions.

Therefore, the University of Copenhagen and the Danish 3R-Center decided to jointly purchase such an artificial horse to be used at the University of Copenhagen (Large Animal Teaching Hospital). The horse arrived at the University from the manufacturing site in Canada in November 2017 and has just been put into use.

Previously, students have made around 600 rectal examinations a year on the university's ten teaching horses. The new artificial horses make it possible to reduce this number to around 100. Due to the many examinations, many of the horses have suffered from minor bleeding around the rectum, and the anaesthetic can also cause the horses to become constipated.

The artificial horse has a wide range of possible uses, both for rectal examinations and in intramuscular and intravenous inoculation exercises.

Asger Lundorff Jensen, Head of Department, Department of Veterinary Clinical Sciences, is pleased with the new model horse. "We've

*already minimized the number of rectal examinations on our laboratory horses out of consideration for the horses. Rectal-examination training is an essential element of the veterinary study programme and, thanks to the new model horse, our students can be better prepared before being allowed to perform a rectal examination on a live laboratory animal, as being better prepared results in a faster, gentler examination,"* Asger explains.

He also believes that the artificial horse gives particularly the youngest students the experience they need before they have to examine a live horse.

*"It can be quite overwhelming for young veterinary students to deal with the first time they have to examine one of our laboratory horses. The artificial horse enables them to be better prepared before they are allowed to try examining a laboratory horse – the real thing. The model horse lets them focus on recognizing the anatomy, learning the technique without having to consider whether their unpractised hand is discomforting or distressful for the animal,"* Asger Lundorff Jensen says.

Later on, the students must try to examine a live horse, under expert guidance, which is why it is still necessary to perform a small number of exploratory examinations on real horses.

See also: <http://vetsimulators.com/products/equine-colic-simulator/>



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*The artificial horse enables them to be better prepared before they are allowed to try examining a laboratory horse – the real thing.*

A large, bold white number '2' is positioned on the right side of the page against a solid teal background. The left side of the page features a photograph of sheep, with one sheep in the foreground looking towards the camera and another in the background. The photograph is partially obscured by the teal background.

# 2

PHOTO: THE DANISH 3R-CENTER



## DISSEMINATION OF INFORMATION

The Danish 3R-Center prioritized its information-related obligations again in 2017. In addition to the centre's continued focus on its website, the board, like last year, chose to give high priority to Science Calling, and – in an effort to reach a “new audience” – the centre experimented with the holding of a popular science lecture at a pub in Aarhus (inspired by the British NC3R's “Pint of Science”) dealing with laboratory animals and the 3Rs.

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## WEBSITE 3RCENTER.DK

Ever since the Danish 3R-Center was formed, the centre has wanted to create a platform where the entire animal-experimentation and alternative milieu could stay up to date on the latest 3R news – an area that will be further prioritized in 2018, by the way. The constant prioritization of the website appears to pay off in terms of site visits with a total of 50% more page views in 2017 compared to the previous year.

The website is not only intended for people in the above-mentioned laboratory animal and alternative milieu, but it is also a venue where the Danish 3R-Center seeks to disseminate knowledge to people outside this milieu, primarily lower-secondary and upper secondary students, because our future scientists and potential users of laboratory animals are found among young people. The website is a means for introducing them to the 3R concept early on.

The Danish 3R-Center also pursues a general education agenda because the centre is interested in ensuring that knowledge of laboratory animals is based on factual

information, thereby providing an alternative to much of the information found on the internet that is either untrue or heavily opinionated.

For this reason, the website project is two-tiered, as the information targeting the laboratory animal and alternative milieu is not interesting to students and vice versa.

The laboratory animal and alternative milieu comprises the intended recipients of news (front page) and of information about research funding and research projects, scientific articles about laboratory animals, presentations from symposiums held, minutes of board meetings, etc.

People outside the milieu comprise the intended recipients of teaching materials (drawn up for both lower secondary and upper secondary schools) and much of the general information about laboratory animals. Due to the Danish 3R-Center's cooperation with the Animal Experiments Inspectorate, the centre possesses all the statistical data about laboratory animals in Denmark, which enables the centre to publish all the information it deems relevant concerning number of laboratory animals, species, severity classifications, etc. These efforts have resulted in a number of visits to the Danish 3R-Center's administration offices by lower secondary students who have chosen to focus on laboratory animals in school and are looking for more details about the use of laboratory animals and the centre's work.

You can sign up for the Danish 3R-Center's newsletter so you do not miss out on information about research funding, 3R events and news from the 3R world (the newsletter is issued about five times a year). Sign up on: [3rcenter.dk](http://3rcenter.dk)



PHOTO: THE DANISH 3R-CENTER

## SCIENCE CALLING

*Science Calling* is a lecture scheme under which scientists, students and others involved with science, technology and health visit schools to tell their own science story.

Peter Bollen, board member for the Danish 3R-Center, offers the lecture *“Animal Experimentation and Alternatives to Animal Experimentation (Dyreforsøg og Alternativer til Dyreforsøg)”*, which took him to six schools (lower secondary schools Næsby, Rantzausminde and Grænseegnens Friskole, and upper secondary schools Grindsted and Fjordbakkeskolen, as well as House of Science). Thus, Peter gave his lecture to a total of 750 students.

The lecture deals with topics such as *Danes’ opinions of animal experimentation; the specific animals used in animal experimentation*

*and what they are used for; the 3Rs; the Danish 3R-Center; and alternatives to animal experimentation, illustrated by the development of cell cultures via “organ-on-a-chip” for a future “human-on-a-chip”*. The lecture also encouraged debate based on the question of whether it will be possible to do without laboratory animals in the future.

For the second consecutive year, the Danish 3R-Center has made an apparently popular contribution to *Science Calling*, by reaching a wide swath of both lower secondary and upper secondary students. The students showed great interest in the topic which was definitely reflected in the students’ inquisitiveness after the lecture.

The Danish 3R-Center will certainly consider taking part in next year’s event (2018).

## PINT OF SCIENCE

Inspired by *“Pint of Science”*, an annual science festival where scientists visit pubs throughout the UK to provide insight into what’s happening in the world of science, the Danish 3R-Center decided to try out the format with a single lecture. The centre’s educative efforts to inform the general public are notably important, to ensure that personal opinions of using laboratory animals are based on facts.

This resulted in the Danish 3R-Center holding its own *“Pint of Science”* event at the Sherlock Holmes Pub in Aarhus on 27 November. The lecture was given by Axel Kornerup Hansen,

board member for the Danish 3R-Center and a professor in laboratory animal welfare at the University of Copenhagen. He explained why it is necessary to use laboratory animals in Denmark and what can be done to make animal experimentation gentler and perhaps someday completely replace it with animal-free methods.

Afterwards, the 25 attendees, primarily students, had an opportunity to ask questions. Due to the great interest shown by the audience, similar events are being considered for 2018.

# 3

PHOTO: KIM GRANLI

# EVENTS

To give an impression of national and international 3R developments, this section presents some of the events that have either been attended by representatives of the Danish 3R-Center or organized by the centre itself.

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## ANNUAL MEETING FOR ANIMAL WELFARE BODIES APRIL

Each year, the *National Committee for the Protection of Animals used for Scientific Purposes* organizes a meeting for animal welfare bodies to discuss the function of these bodies. The meetings have successfully been expanded so that the programme for the animal welfare bodies takes place before noon, and the nature of the meeting changes in the afternoon to become an inspiration event offering networking for everyone with a professional interest in laboratory animals. The fact that all of 41 of Denmark's 47 animal welfare bodies took part in the meeting is visible proof of the event's success and relevance.

The annual meeting was held at the *Centre for Health and Society, University of Copenhagen*, and focused on how to heighten awareness of the animal welfare body to all relevant persons in an organization for the benefit of the laboratory animals.

Awareness can be heightened by each animal welfare body reaching out to those who are involved in animal experimentation at the respective facilities, e.g. by means of information sessions or by offering relevant training courses.

The programme of events also featured Leif Røge Lund and Kirsten Bayer Andersen, representing the Animal Experiments Inspectorate. They started out by telling about how the Inspectorate processes applications for animal experimentation, followed by a description of the problems they sometimes experience in Denmark's laboratory-animal housing units during Inspectorate inspections.

The final part of the programme (networking and inspiration) was also very successful. It not only attracts many interested participants, but many of them also bring and display their 3R initiatives which in one way or another have had a positive effect on animal experimentation at their respective institution/company. They exhibit their initiatives and visitors move from one stand to another to hear about the objects and ideas brought to the event. (The above-mentioned initiatives can be found on the committee's website: go to [www.fvst.dk](http://www.fvst.dk) and search for the National Committee for the Protection of Animals used for Scientific Purposes.)

Last, but not least, the winners of the competition for paraveterinary workers were announced.

In the time leading up to the meeting, it was possible to submit ideas for promoting the welfare of laboratory animals to the National Committee for the Protection of Animals used for Scientific Purposes, which subsequently assessed the submitted proposals.

The winners of the 3R competition were Heidi Lehman, Josefine Hammer and Janni Oxfeldt from the National Veterinary Institute at Lindholm, for their proposals for environmental enrichment (refinement) for minks used as laboratory animals. The National Committee for the Protection of Animals used for Scientific Purposes awarded them with DKK 25,000 for their Refinement initiative.



## THE NATIONAL COMMITTEE FOR THE PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

The Committee is set up to advise the appropriate body (in Denmark: the Danish Animal Experimentation Inspectorate) and animal welfare bodies in matters relating to the acquisition, breeding, accommodation, care and use of laboratory animals and to facilitate the sharing of best practices. All animal experimentation facilities must be affiliated with an animal welfare body tasked with providing advice to scientists, animal keepers, veterinarians and other professionals on the use of the 3Rs in research. The Committee also aims to ensure the sharing of 3R knowledge with committees from other EU member states.

The committee's members are also members of the board of the Danish 3R-Center.

PHOTO: THE DANISH 3R-CENTER

### ANIMAL WELFARE BODY: AN ANIMAL WELFARE BODY HAS THE FOLLOWING TASKS:

- provide advice on animal welfare issues relating to acquisition, accommodation, care and use;
- provide advice on how to apply the principles of replacement, reduction and refinement;
- provide information about technological and scientific developments in replacement, reduction and refinement;
- define and update internal operating procedures with respect to monitoring, reporting and follow-up concerning the welfare of animals housed or used in the institution or company;
- monitor the development and results of projects with due consideration of the effect on the animals used and identify and advise on any factors that can further contribute to replacement, reduction and refinement; and
- provide advice on plans for rehabilitation, including socialization, of animals that need to be moved to a new location or returned to a habitat or production system suitable for the species

# 2018 ANNUAL MEETING

## ANNUAL MEETING OF THE ANIMAL WELFARE BODIES

The National Committee for the Protection of Animals used for Scientific Purposes invites animal welfare bodies and stakeholders to an annual meeting every year. Further details are available on the Committee's website [www.fvst.dk](http://www.fvst.dk), search for the **National Committee for the Protection of Animals used for Scientific Purposes**.

7 JUNI 2018 AT THE FACULTY OF HEALTH AND  
MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN,  
ØSTER FARIMAGSGADE 5, 1353 COPENHAGEN K

## 3R COMPETITION FOR **PARAVETERINARY STAFF**

The National Committee for the Protection of Animals used for Scientific Purposes sponsors a competition where it is possible to win up to DKK 25,000.

Do you have any ideas about how to improve the welfare of laboratory animals at your facility? Would you like to try out a new type of refinement, a new method or a completely different 3R initiative? We frequently encounter paraveterinary staff who have good ideas for 3R initiatives. Insufficient funds are often cited as the main reason that these ideas are not implemented.

Therefore, the National Committee for the Protection of Animals used for Scientific Purposes sponsors a competition where it is possible to win up to DKK 25,000 to initiate or try out a 3R initiative or 3R idea. A brief written application is sent to the National Committee for the Protection of Animals used for Scientific Purposes, after which the Committee selects which proposal(s) to support. The winner of the competition is presented during the open segment of the annual meeting for Animal Welfare Bodies on 7 June 2018.

When the project is completed, the project participants must present the results in a report submitted to the Committee and at the 2018 Annual Meeting for Animal Welfare Bodies. Send project proposals and budget to [ufa@fvst.dk](mailto:ufa@fvst.dk). The person responsible for financial affairs at the experiment facility must sign the application.

**The competition deadline is 22 May 2018.**

# FIRST-FLOOR PLAYGROUND

We turn over the floor to the winners of the 2017 3R Award for paraveterinary staff (January 2018).



PHOTO: THE DANISH 3R-CENTER

The animal experimentation facility at Lindholm (National Veterinary Institute) won the 3R competition in 2017 sponsored by the National Committee for the Protection of Animals used for Scientific Purposes, which enabled us to launch an animal-welfare initiative aimed at improving and refining the housing environment for laboratory mink. We dubbed the project “First Floor Playground” (Legeplads på 1. sal), and the idea was to provide our mink with a more complex and active environment. It is crucial for mink well-being and welfare that they be given access to a stimulating environment which encourages activity and which challenges their curiosity during their active hours. The project tried to optimize the cage environment in two ways: 1) by devising a more complex cage structure/ multilevel cage with access to an upper floor via a plastic hatch; and 2) by adding equipment and toys to the first floor which stimulate natural behaviour.

Heidi Lehman, initiator of the 3R project, said: *“We’ve completed most of the things that we had planned to test on the minks. We’ve learned more about what works well and what doesn’t. We bought new multi-level cages and new pup netting that we laid on the cage bottom to relieve pressure bruises on paws. We tried out lots of different toys and different types of materials for rooting and bathing.”*

*“The first thing we tried was to give the minks a container of water so they could bathe. It was very successful, and it subsequently turned out to be the initiative that worked best for both the minks and for cleaning/handling by the animal keepers.”*

*“We also tried out sand and wood shavings – the minks also liked it, but several of them started using the material as a toilet, which was not so good.”*

*“We added different types of toys/enrichment to the water tub: meal worms/dried fish, which the minks could dive for and plastic balls/wooden rings that could float on top of the water. It soon turned out that the toys had to be fixed, however because otherwise they would get “captured” and taken into the nesting box, and they would lose interest in it. So, after suspending items in the upper cage, the minks spent lots of time with the toys – a half-litre plastic bottle with a little water in it was particularly inspiring for play.”*

*“We’ve experienced the minks as being calmer and less stressed. We haven’t observed stereotypical behaviour after starting the 3R project.”*

*“The conclusion from our current experience is that the water bath works all the time and that the toys have to be regularly replaced to maintain interest.”*

*“We now have the opportunity to set up as many as 100 animal-welfare cages for mink procedures, and we’re planning to have all future experiments involving mink take place in the enriched housing environment.”*

*“It was an interesting, educational project and we definitely recommend others to take the plunge and do it if you can. It is a source of great delight for both animals and keepers.”*

The project will be presented in more detail at the Annual Meeting for Animal Welfare Bodies on 7 June 2018.

# WORLD DAY FOR LABORATORY ANIMALS

## 24 APRIL

For the sixth consecutive year in Denmark, the Danish Animal Welfare Society (DAWS) and DOSO organized the World Day for Laboratory Animals that highlights current topics and issues related to the use of laboratory animals.

This year's theme was "Experiments using Farm Animals – the related application of the 3Rs", and the programme included talks by the Danish Animal Welfare Society, DOSO, the Animal Experiments Inspectorate, Aarhus University, Novo Nordisk, Vilvorde (Roskilde Technical School), Aalborg University Hospital, Ellegaard Göttingen Minipigs A/S, University of Copenhagen and Aalborg University.

The programme is also a compulsory part of the study programme for veterinary medicine students who choose to specialize in laboratory animals and animal experimentation during their final year of study.

The Danish 3R-Center was indirectly represented on the programme. Tom Bengtsen, Head of the Secretariat for both the Danish 3R-Center and the Animal Experiments Inspectorate was invited in his latter capacity to talk about the Animal Experiments Inspectorate's topical initiatives, including the current situation of using farm animals as laboratory animals. Tom told how the number of laboratory animals in Denmark in 2015 (latest figures) was about 240,000, by and large identical with previous years. Out of this number, more than 80% are mice and rats.

Conventional farm animals constitute only a small percentage of the total number of laboratory animals. In 2015, just over 30,000 cattle, pigs, horses, sheep, goats, poultry and fish were used. Most farm animals are

used for basic research or applied research, and quite a large number are also used for teaching purposes. This takes place either as part of agricultural or veterinary medicine study programmes or as models for humans.

The programme included a wide range of interesting presentations related to the 3Rs and animal welfare, a few of which are mentioned below.

One was Cathrine Juel Bundgaard's presentation about the practical application of the 3Rs to procedures using pigs at Novo Nordisk. Her talk provided insight into some of the practical measures implemented at Novo Nordisk to maintain focus on the 3Rs in every aspect of pig procedures (and in procedures involving other laboratory animals), in the planning phase, in housing the animals and while procedures are being conducted.

Cathrine also described how Novo Nordisk, like the Danish 3R-Center, awards a 3R prize every year to employees who have made an outstanding achievement relating to the 3Rs – adding that many projects are submitted for assessment every year.

Another talk was held by Jens Ellegaard, owner of Ellegaard Göttingen Minipigs (Ellegaard evolved from a traditional pig farm back in the 1970s and has been breeding minipigs as laboratory animals since 1982). The company continuously works to improve minipig health and welfare in the way the minipigs are housed and transported. Ellegaard works closely together with its customers to ensure the best possible care, accommodation and handling of the minipigs at the premises of its customers to ensure good minipig welfare and minimize stress in both animals and keepers at the same time.

Different types of enrichment of the pigs' environment, socialization, their activation and training all help to improve their animal welfare. The training of customers' staff is also an important aspect related to animal welfare.

As a new initiative, DOSO and the Danish Animal Welfare Society, in collaboration with the Danish Association of the Pharmaceutical Industry (Lif), awarded a prize earmarked for an animal keeper, laboratory technician or paraveterinary worker who has made an extraordinary effort to improve animal welfare or disseminate knowledge about the 3Rs.

The winner of the 2017 3R Award was Annett Christoffersen (Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen). In the

assessment, DOSO and the Danish Animal Welfare Society attached great importance to the fact that Annett had shown independent initiative and had developed her own Refinement measures at her workplace. Annett has an excellent understanding of the animals' internal hierarchy and relationships, which enabled her to help prevent the "bullying" of animals further down the totem pole, which is presumably a somewhat overlooked welfare problem.

In conclusion, let us pause to consider the impressive fact that it is possible in Denmark to hold a constructive seminar revolving around laboratory animals – a topic considered controversial in many countries – with representatives from animal welfare organizations, business and industry and universities.

## SCANDINAVIAN SOCIETY FOR LABORATORY ANIMAL SCIENCE SCAND-LAS, 30 MAY-2 JUNE

In 2017, Scand-LAS' annual meeting was held in Copenhagen. The topic of the meeting was "Do we need animal experimentation?". The meeting was introduced by two lectures on ethics relating to the use of animals for scientific purposes: Peter Singers, author of the book *Animal Liberation* which is widely considered the bible of the animal rights movement, spoke about "Speciesism", and Mickey Gjerris gave a presentation entitled "The Lesser Evil".

Several speakers rounded off their presentations by trying to answer the question of whether animal experimentation is still necessary: most answered them answered

with a "Yes, but...", thus expressing that we are unfortunately unable to phase out the use of laboratory animals at present, but that we must continue to ensure that animal-free methods are used if viable alternatives exist.

The Danish 3R-Center's board and secretariat were represented at the meeting. In addition to technical content, the meeting was as usual a good forum in which to meet our stakeholders.

In 2018, the annual meeting of Scand-LAS will be held in Kristiansand, Norway on 26-28 April. The topic of the meeting is *Beyond legislation – Best practice in animal research*.



# 10<sup>TH</sup> WORLD CONGRESS ON **ALTERNATIVES AND ANIMAL USE** IN THE LIFE SCIENCES 20-24 AUGUST

Representatives from the Danish 3R-Center's board and secretariat took part in the *World Congress on Alternatives and Animal Use in the Life Sciences*. The World Congress takes place every three years and the 2017 congress was the tenth in the series (the first being held in Baltimore, USA, in 1993), held in turns in Europe and outside of Europe.

The Congress's theme was *The Three Rs in Action* and included a total of 800 lectures within nine themes (see below). The World Congress is the biggest, most important event in the 3R world in every way, which is why the Danish 3R-Center gives high priority to participating. The World Congress is attended by almost 1,000 participants from roughly 50 countries, providing a good opportunity to be inspired in a wide variety of areas.

## CONGRESS THEMES

### ETHICS

Sessions in this theme will explore the current thinking surrounding harm/benefit analysis, role of ethics committees/IACUCs in promoting ethical research, ethics of using certain species for research, and ethical challenges presented by new technologies.

### LESSONS LEARNED

Sessions will highlight regulatory acceptance of replacement methods in the US and Europe, the acceptance of replacement methods for investigative new drug applications, the use of virtual screening and read across methods and integration of *in vitro* methods for organ toxicity screening, and novel and upcoming possibilities for incorporating *in vitro* methods for safety and toxicity testing.

### INNOVATIVE MODELS FOR SAFETY AND EFFICACY

There are new and emerging methods that are being developed or used in chemical evaluation as models of human disease. Sessions in this theme will be organized around organ systems and disease models and will include talks on basic physiology, talks on disease states as well as applications including translation to novel adverse outcome pathways (AOPs) and or Mechanisms of Action (MOAs). Computational toxicology approaches will also be addressed within the model discussions. This theme will include sessions on metabolism and kinetic models to link outcomes for translation. Exposure considerations for these models will also be included as will review of models for evaluating drugs, environmental agents, vaccines and medical devices for safety and efficacy.

## SUSTAINABILITY

This theme will focus on more sustainable methods research and development. Green chemistry will include the development and application of new tools, technologies and approaches to guide the design and use of inherently safer chemicals, such as through the development, acceptance and implementation of new computer- and cell-based 21st century technologies. One Health will address efforts to practice and promote proper stewardship of biological and animal resources, whether through conscientious use of antibiotics, the development of sustainable agricultural.

## SYSTEMS BIOLOGY AND BIG DATA

This theme will address challenges and emerging solutions for big data to support systems biology for 3Rs with regards to scientific empiricism, theory, simulation and prediction: what are the resources available now to deal with the surfeit of biological data and information; what are the pragmatic approaches for visualization; and how do we prepare for the challenges of bigger data in the future.practices, or the use of naturally occurring disease models in place of traditional laboratory animals.

## 3RS IN ACADEMIA

This theme will address the use of animals and new non-animal technologies in education, the communication of the 3Rs information and its implementation around the world, incorporating the 3Rs into basic research and funding for 3Rs.

## TRANSLATION

This theme encompasses best practices and mechanisms (including validation) to achieve optimal translation of research into practice. Topics will include biomarkers, relevance of animal models of human disease, implications of systematic review of animal and non-animal methods and best practices for various replacement alternative methods.

## REFINEMENT AND ANIMAL WELFARE

In this theme, sessions will cover the use of technology to improve animal welfare, advanced methods for recognition and alleviation of pain in laboratory animals, how to achieve better reproducibility in animal experiments, and welfare concerns about genetically modified animals.

## GLOBAL COOPERATION

This theme will address efforts around the world to harmonize methods and validation efforts, the role of international science in promoting the 3Rs, role of advocacy groups in promoting the 3Rs globally and international cooperation.



PHOTO: THE DANISH 3R-CENTER

## Networking

In the view of the Danish 3R-Center, the Congress is rewarding for several reasons. First of all, it is important in terms of networking, because the Danish 3R-Center can draw attention to itself and its work. For instance, it was at the World Congress that the Danish 3R-Center and the recently formed Swedish 3Rs Center engaged in initial discussions about future cooperation. These discussions resulted in the Swedish centre participating in the Danish 3R-Center's symposium (7–8 November) and the Danish 3R-Center participating in the official opening of the Swedish 3Rs Center (21 November).

For the Congress's numerous poster sessions (350 posters were displayed at the Congress), the Danish 3R-Center's representatives had brought a poster about the Danish 3R-Center and the National Committee for the Protection of Animals used for Scientific Purposes, and the poster attracted great interest in the work being conducted under the auspices of both bodies.

Especially the Danish 3R-Center's recently published teaching materials on laboratory animals and 3R aroused interest, and the possibility of translating the material into English (or other languages) was discussed. The possibility of a translation was discussed with, among others, a representative from Canada's Animals in Science. They are working to build up a science culture that respects animal life by promoting the reduction and replacement of animals in education, research and testing, which is definitely a mission that the Danish 3R-Center can envision pursuing and explains why this type of cooperation is possible.

Other visitors perused the poster from the National Committee for the Protection of Animals used for Scientific Purposes which presented the annual meeting for Denmark's animal welfare bodies (described previously in the annual report). Especially the idea of presenting a 3R initiative (usually Refinement)

at the annual meeting aroused interest. Spreading the word about the annual meetings – and the 3R ideas – is the very purpose of the poster, and it will definitely be positive if others are inspired by the concept.

When the Danish 3R-Center's representatives took turns standing by the posters they had brought to the event, it also provided an opportunity to advertise the Danish 3R-Center's symposium (held in English), which could be of interest to many of the same individuals and organizations attending the World Congress. This year, 2018, the Danish 3R-Center will focus on increasing the number of non-Danish attendees at the symposium, not only to raise awareness about the Danish 3R-Center and its work, but also to enable foreign visitors and lecturers to impart new knowledge to Danish visitors, which could perhaps result in international cooperation to boot.

The World Congress also provided an opportunity to cultivate familiar contacts, such as the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and the Canadian Council on Animal Care (CCAC).

## The North American 3Rs Collaborative (NA3RsC.org)

Another interesting initiative was presented at the Congress: The North American 3Rs Collaborative (NA3RsC.org). It seeks to promote the 3Rs by enhancing communication, knowledge-sharing and cooperation among 3R organizations and the world of research, also in international respects.

To achieve this, they have created a virtual 3R site. The website contains a wide range of interesting initiatives, such as a lecture hall where virtual symposiums can be held; a room where 3R organizations can present themselves at virtual stands; a discussion forum; a 3R resource centre, etc. – all of which are designed to unite the 3R world even more.

The Danish 3R-Center is closely monitoring this initiative and will try to upload some relevant information about the Danish 3R-Center and information with 3R contents deemed relevant to website visitors.

### Inspiration

The Danish 3R-Center also attends the World Congress to seek inspiration for the centre's own symposium in terms of interesting speakers or topics. For instance,

it was at the World Congress that we noticed Fawzy Elnady, Cairo University, who held a lecture entitled Use of the Elnady Technique for preserving specimens in education and training (the lecture is described in the section on the Danish 3R-Center's symposium).

Later, Fawzy Elnady also talked about a certain progress in Africa in the 3R area. For instance, most universities with activities involving animals have established ethical committees.

## 11<sup>TH</sup> WORLD CONGRESSES ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES

23-27 AUGUST 2020

**3Rs in transition**  
– from development  
to application

WC11MAASTRICHT.ORG

## NORECOPAS 10TH ANNIVERSARY 10 OCTOBER



PHOTO: THE DANISH 3R-CENTER

Norecopa ([norecopa.no](http://norecopa.no)) celebrated its tenth anniversary in Oslo on 10 October 2017. Like the Danish 3R-Center, Norecopa works to promote the 3Rs.

Bente Bergersen, Chairwoman of Norecopa's board, welcomed everyone to the event, after which Adrian Smith, Head of the Secretariat and member of the board of the Danish 3R-Center, presented an overview of Norecopa's brilliant work over the past decade. A document about this has also been published: *Norecopa: The first ten years* ([norecopa.no/about-norecopa/the-first-10-years](http://norecopa.no/about-norecopa/the-first-10-years)).

Adrian Smith presented Norecopa's current guidelines for planning animal experimentation (PREPARE), which were published in August 2017 (further details in Chapter 5).

This was followed by a series of presentations from external lecturers about current research and 3R topics. Leopold Koenig, TissUse, Berlin, described his organization's work involving *humans-on-a-chip* – i.e. *in vitro* methods which combine micro-chip technology and human cells.

Tom Bengtson, Head of Secretariat, the Danish 3R-Center, presented the centre's work, and articulated the centre's visions for future

cooperation between the Nordic 3R centres where the centres can cooperate particularly in the field of research by communicating and "selling" one another's products, i.e. the Danish 3R-Center's research results and teaching materials, Norecopa's PREPARE guidelines and 3R database, etc.

John Linnell, Norwegian Institute for Nature Research (NINA) illustrated the ethical aspects of research using feral animals and the relationships between field experiments and efforts to preserve biodiversity.

After this, it was time for Gøril Eide Flaten, one of the winners of Norecopa's 3R award. She presented her work involving the development of an *in vitro* technique for studying the absorption of medicines in the intestines.

Finally, Elliot Lilley of *The Royal Society for the Prevention of Cruelty to Animals* (RSPCA) presented his viewpoints on the positive aspects that the 3Rs have contributed to the laboratory animal environment. In this context, Elliot mentioned the numerous scientific conferences and task forces that, like the 3Rs, contribute a common frame of reference to the laboratory animal environment, which helps improve animal welfare.



## THE DANISH 3R-CENTER'S SYMPOSIUM 7-8 NOVEMBER

An important part of the Danish 3R-Center's work is the annual holding of an international symposium. The symposium provides an opportunity for the centre to tell about its work over the year and it gives researchers financially supported by the centre a platform for disseminating their 3R project results to the laboratory animal and alternative milieu. The Danish 3R-Center also invites a number of national and international personalities with 3R expertise to talk about their sphere of work.

This year's symposium was held in Copenhagen with just short of 200 attendees interested

in laboratory animals and alternatives in one form or another (researchers, students, paraveterinary workers, animal keepers, animal welfare organization representatives, etc.).

The year's conference was moderated by Adrian Smith, member of the board of the Danish 3R-Center. Adrian introduced the symposium by providing practical information before turning over the floor to Christine Nellemann, Chairwoman of the board of the Danish 3R-Center, and the first speaker at the symposium.



PHOTO: KIM GRANLID



PHOTO: KIM GRANLI

## NOVEMBER 2017

# THE DANISH 3R-CENTER - ACHIEVEMENTS 2013-2017 AND FUTURE TASKS

**Christine Nellemann**, *the Danish 3R-Center*

Christine Nellemann provided an update on the current situation for the Danish 3R-Center's work during its first four years of existence, describing the many initiatives of the centre to promote the 3Rs in Denmark. As the first (four-year) term of the board of the Danish 3R-Center had just expired, it seemed like a good time to take stock of the situation.

Christine started by outlining the four 3R areas on which the centre concentrates its activities.

1. Collecting and disseminating information
2. Initiating and supporting research
3. Hosting an annual international symposium
4. International cooperation

In the area of collecting and disseminating information, Christine described the centre's efforts with its website, newsletters, publication of statistical information on the use of laboratory animals in Denmark on the website, the centre's teaching materials about laboratory animals and the 3Rs, and the annual report, as well as "Science Calling" where board members visit a number of schools to provide insight into the world of laboratory animals. Christine expressed her great satisfaction with the efforts made in this area, which, among other things, have steadily increased the

number of visitors to the website and resulted in a large number of subscribers to the newsletter. Christine was also pleased by the fact that upper secondary schools are starting to use the teaching materials.

Christine explained how the centre has supported thirteen 3R projects over its first four years, all of which make a positive contribution to optimizing the laboratory animal area in Denmark.

In addition, the Danish 3R-Center has taken the initiative to launch three of its own projects, one of which is finished and two of which are being prepared. The first-mentioned was a study from 2016 dealing with charting the knowledge, attitudes and experiences of Danish users of laboratory animals relating to the 3Rs. The study was conducted by Rikke Nøhr, Thomas Bøker Lund and Jesper Lassen from the University of Copenhagen.

The survey resulted in the report *The Danish 3R survey: knowledge, attitudes and experiences with the 3Rs among researchers involved in animal experiments in Denmark*, which is published on the website of the University of Copenhagen (see the summary of the report on the next page).

*Summary***The Danish 3R survey: Knowledge, attitudes and experiences with the 3Rs among researchers involved in animal experiments in Denmark**

The purpose of the Danish 3R study (2016) was to study Danish laboratory-animal researchers' knowledge of and lessons learnt from the 3Rs. The purpose was also to make a qualitative survey of researchers' expectations of the Danish 3R-Center. A total of 293 animal experimentation researchers responded to the questionnaire developed for this purpose.

Most of the animal experimentation researchers personally assessed that they were very knowledgeable of the 3Rs. Similarly, the study showed that the researchers also have a relatively high level of factual knowledge. The responses showed that researchers' knowledge of reduction and replacement is greater than their knowledge of refinement, which is slightly less.

The qualitative survey of researchers' specific lessons learnt from implementing the 3Rs showed that a vast majority perceive that refinement and reduction play an important part in their planning and implementation of animal experiments. Similarly, many indicated that they had developed techniques aimed at achieving both refinement and reduction. The study also showed that replacement plays a certain part in the planning and design of experiments, although it is unclear how this is done in practice.

A vast majority of the researchers who took part in the survey do not think that implementing the 3Rs will undermine their work, nor do many see any barriers to the implementation of the three principles. In spite of this, only a minority think that the objective of complete replacement will be achieved and they doubt that the use of animal testing will be completely eliminated.

When asked directly about the barriers to further implementation of the 3Rs, many

emphasized the lack of relevant research and innovation as a significant factor. They also singled out increased data sharing and a higher degree of cooperation among researchers as factors that could promote the implementation of reduction. Similarly, scientific factors, such as better accessibility of human tissue, a greater number of relevant cell cultures and better computer models were identified as the most important conditions for increasing replacement.

The Danish 3R-Center also turned out to be relatively well-known among researchers, the majority of whom think that the centre has an important role to play for researchers working with laboratory animals in Denmark.

Overall, the survey showed significant differences between researchers, depending on whether they are employed in the public or private sector. For instance, private-sector researchers assess their own level of knowledge to be higher and they also seemed to have a better factual understanding of the three principles. The survey also identified a significant difference between the two groups' awareness of the 3Rs. Private-sector researchers primarily consider the 3Rs during their daily work with animals, for instance, whereas public-sector researchers primarily considered the 3Rs when they were designing projects or writing applications for approval.

The survey singled out a number of factors that the Danish 3R-Center is advised to consider in its work going forward. It advises the centre to employ a two-tiered strategy which, on the one hand, focuses on aiming 3R information at public-sector laboratory-animal researchers where there is deemed to be a particular need for further knowledge that could help make the 3Rs operational in their day-to-day research involving animals. On the other hand, efforts should be made to

continue forming networks – as recommended – such as at conference and seminar events, and at the annual 3R symposium.

The second project launched by the centre (which is still in process as of January 2018) is the study *Replacement – options and challenges*, and it is also being conducted by the University of Copenhagen. Christine told how the purpose of the study is to examine the opportunities and challenges relating to the implementation of alternatives (replacement) at research facilities in the private and public sectors. The study results are expected to be published in the spring of 2018.

The third project described by Christine deals with *Severe Suffering*, where the Danish 3R-Center wishes to explore the possibility of reducing the number of laboratory animals in Denmark subjected to procedures involving

severe suffering. (As the project is described in Chapter 1, it will not be further discussed here.)

The third main area in which the centre is involved deals with the planning of the annual international symposium. Christine expressed great satisfaction with the progress made by the symposium since 2014, particularly citing the satisfaction of experiencing the steadily increasing number of participants from both Denmark and abroad.

In her concluding remarks, Christine touched on the centre's wish to cooperate with international 3R organizations, which is why centre representatives frequently take part in international meetings and conferences, both to draw attention to the centre and its work but also to make contact for the purpose of cooperating in the 3R area.

## USE OF THE **ELNADY TECHNIQUE** FOR PRESERVING SPECIMENS IN EDUCATION AND TRAINING

**Fawzy Elnady**, *Cairo University*

The next speaker was professor Fawzy Elnady, whose lecture had been heard by representatives of the Danish 3R-Center attending the *10th World Congress on Alternatives and Animal Use in the Life Sciences* (August 2017). Due to the unique topic of his lecture, they chose to invite him to speak at the Danish 3R symposium.

Fawzy Elnady has developed his own method of preserving animals and organs, which can be used as an alternative, e.g. in veterinary medicine study programmes, and it could spare a large number of animals as the specimens can be used for many years. According to Fawzy Elnady, the specimens have an advantage, compared to many other alternatives for teaching use – e.g. software and virtual reality, mannequins and plasticized specimens. All of these are viable, usable alternatives, but they

do not provide sufficient hands-on experience, as Elnady's specimens are realistic, dry, durable, flexible and inexpensive to make.

Fawzy Elnady explained how his specimens had many potential uses. Organs and entire bodies can be developed for basic anatomy and dissection. For clinical skills, the technique has been successfully used in surgical suturing of wounds, endoscopy of the upper respiratory tract in horses, dystocia (obstructed labour) in cows and mares – to mention just a few. The technique can also support examinations in the areas of embryology, pathology, parasitology and internal medicine.

Fawzy had brought samples of anatomical specimens, which he presented at a stand in the rear of the lecture hall, which attracted great interest.

The following lectures are not included here, as the projects are described elsewhere in this annual report:

Developing facts-based in vitro assays to measure antibody mediated protection against infection with intracellular bacteria (3R project supported in 2017)

**Jes Dietrich**, *Statens Serum Institut*

Implementation of analgesic refinement in rats used as models for arthritis and inflammatory pain (3R project supported in 2017)

**Klas Abelson**, *University of Copenhagen*

Equine palpation/colic simulator

**Asger Lundorff Jensen**,  
*University of Copenhagen*

Towards better brain cancer treatment with novel in vitro models and fewer animal experiments (3R project supported in 2016)

**Bjarne Winther Christensen**,  
*University of Southern Denmark*

Development of computer models to predict chemicals interference with thyroid hormones (3R project supported in 2016)

**Marianne Dybdahl**, *Technical University of Denmark*

Use of cell culture technology to minimize the needs for animal trials in development and production of fish vaccines (3R project supported in 2016)

**Niels Lorenzen**, *Aarhus University*

Establishment of an *in vitro* model for diabetic atherosclerosis (3R project supported in 2014)

**Mette Bjerre**, *Aarhus Universitet*

A refined approach to producing polyclonal antibodies in chickens – completely replacing all invasive elements by combining immunizations with routine aerosol-based vaccinations (3R project supported in 2015)

**Otto Kalliokoski**, *Københavns Universitet*

An alternative to animal experiments: development of an *in vitro* human skin model for evaluation of topical antimicrobial compounds (3R project supported in 2015)

**June Lissa Hansen**, *Rigshospitalet*





PHOTO: KIM GRANLI

## INTRODUCING THE NEW SWEDISH 3RS CENTER

**Torsten Jakobsson**, *Swedish 3Rs Center*

The next speaker was veterinarian Torsten Jakobsson, who has spent the past year organizing the Swedish 3Rs Center. Torsten started by describing how the centre is placed under the Swedish Board of Agriculture and that its future work will be conducted under the motto “Animal Welfare in Focus and Reduction of Animals in Research”.

In Sweden, the Swedish National Committee for the Protection of Animals used for Scientific Purposes has been made the steering committee for the Swedish 3Rs Center. Accordingly, the committee’s role can be compared to the role of the board of the Danish 3R-Center, and the Swedish 3Rs Center’s role is comparable to the part played by the secretariat of the Danish 3R-Center. In other

words, the Swedish 3Rs Center will serve as the executive segment of its own organization.

Torsten also presented a list of the tasks charged to the Swedish 3Rs Center: providing consultation and support to ethical committees; serving as an information centre for alternatives (replacement); working on best practices at national and international levels; cooperating with animal welfare bodies; supporting the sharing of organs and tissue – and much more besides.

Obviously, the lecture was marked by the fact that the Swedish 3Rs Center is in a start-up phase and actually opened officially ten days after the Danish symposium (an event described in Chapter 5 of the annual report).

# THE “6RS” OF ALTERNATIVE METHODS IN THE 21<sup>ST</sup> CENTURY: ADDING RELEVANCE, READ-ACROSS AND ROADMAPS

**Thomas Hartung**, *Johns Hopkins Bloomberg School of Public Health*

To many, Thomas Hartung needs no introduction, as he has been a long-standing key figure in the 3R world, exemplified by the fact that Thomas Hartung managed *The European Union Reference Laboratory for alternatives to animal testing* (EURL-ECVAM) for many years. Thomas Hartung is soundly based in both the European and US toxicology-testing universes by virtue of his participation in an abundance of activities.

Thomas Hartung gave a presentation on the 6Rs, a concept that supplements the “real” 3Rs with three more: Relevance, Read-Across and Roadmaps.

“Relevance” means that researchers must use models which ensure a relevant decision-making basis: the models must be human-oriented by using cell cultures of human origin and by observing good laboratory practices. Mechanism studies are the basis for developing these relevant models and their validation. In this context, sharing data in large, common, publicly accessible databases would be a big step forward. Today, the results of cell-based studies are compared to data from animal experimentation, which makes little sense as animal experimentation does not necessarily predict risk in humans.

“Read-Across” means a simple comparison of the effect of related chemicals, so that a common feature can be identified and used to predict effects. It works from one group of substances to another but cannot be validated per se. And this is where the big shared databases can provide useful background data that can be used by many to replace animal experimentation. Read-Across is a tool developed for acute toxicity testing, irritative effects and mutagenicity.

“Roadmaps” refers to the strategic considerations relating to mechanism-driven toxicology, which in this context focuses on training and communication, as well as systematic reviews and evidence-based toxicology involving all toxicological stakeholders.

Thomas emphasized that animal experimentation is not always predictive of human risk and, seeing that there are hundreds of thousands of chemicals that need to be tested, the use of computer-based solutions and in vitro assays is the way forward.

After Thomas’ presentation, a poster session was held which served simultaneously as a social event with wine and snacks and was an event that many of the symposium attendees choose to take part in. Around twenty posters were presented (they are shown on the Danish 3R-Center’s website), and according to the subsequent satisfaction survey, the symposium visitors were very satisfied particularly with this session’s networking opportunities. However, the Danish 3R-Center will endeavour to ensure that people who bring a poster to the event will have even better possibilities of taking the floor at future symposiums.



PHOTO: KIM GRANLI

## POSTERS AT THE 3R-SYMPOSIUM

[3rcenter.dk/arrangementer/symposium-2017/posters/](http://3rcenter.dk/arrangementer/symposium-2017/posters/)

A new approach to model the initial phase of fracture healing *in vitro*

**Moritz Pfeiffenberger,**  
Charité Universitätsmedizin Berlin

Development of an *in vitro* multi-component 3D joint model

**Alexandra Damerau,**  
Charité Universitätsmedizin Berlin

A real-time *in vitro* assay as a potential predictor of *in vivo* tumour imaging properties

**Jonas Stenberg,**  
Ridgeview Instruments AB

Do modern technologies allow you to perform optimal health monitoring in IVCs? The challenge of balancing 5 pillars – science, cost, risk assessment, management and 3Rs.

**David Mayo,** IDEXX BioResearch EMEA

Adapting pre-clinical systematic review protocols to physiological questions: an approach by AUGUST

**Tristan Hollyer PhD,**  
Aarhus University Hospital

Ferrets as animal models in research: Establishment of an open housing facility and blood sampling technique

**Trine Schütt,** LEO Pharma A/S

Assay of inactivated poliomyelitis vaccine: comparison of *in vitro* vs. *in vivo* assays for potency determination

**Sten Erik Jensen, PhD,**  
Statens Serum Institut

Prediction of acute lung toxicity of impregnation products using an *in vitro* method based on lung surfactant inhibition

**Emilie Da Silva og Jorid Sørli,**  
National Research Centre for the Working Environment

Capillary micro sampling (CMS) techniques in rodents, a reduction and refinement method that delivers high quality data

**Janne Koch,** LEO Pharma A/S

PREPARE before you ARRIVE: Guidelines for Planning Animal Research and Testing

**Norecopa**

Promoting tissue sharing

**Sophie Wiese,**

*Neuro Ecological Research Denmark IVS*

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Reduction of aggression among  
group housed male mice by use of  
talcum

**Jakob le Fèvre Harslund,**

*Aarhus Universitet*

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Simulating osteoarthritis *in vitro*  
with human scaffold-free 3D  
cartilage transplants

**Marie-Christin Weber,**

*University Hospital Berlin*

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Use of cell culture technology to  
minimize the need for animal trials  
in development and production of  
fish vaccines

**Niels Lorenzen et al,**

*Aarhus University et al*

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Use of Primary Isolates From  
Human Kidney to Study the  
Molecular Aspects of Blood  
Pressure Regulation

**Henrik Dimke,**

*University of Southern Denmark*





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# SAFETY IN DRUG DEVELOPMENT: THE LINK BETWEEN HISTORICAL MEDICAL CASES AND CURRENT REGULATIONS

**Zindy Raida**, *Novo Nordisk*

The first speaker on the second day of the symposium was Zindy Raida of Novo Nordisk. Zindy gave a presentation about how medical tragedies have prompted the imposition of strict regulatory requirements on drug development over the years. The purpose of safety pharmacology is to look at potentially undesirable physiological effects of a substance being studied for drug authorization before it is tested on human beings for the first time.

There was no legislation to regulate medicine until 1902. Back then, this meant that drug manufacturers could use the product label to promise that their drug was a cure for almost anything.

In 1901, 13 children died in the Tetanus Scandal in the US after having been given antiserum from a horse that subsequently developed tetanus. This led to the adoption of The Biologics Control Act of 1902 (aka The Virus-Toxin Law). After this, the manufacturer had to specify information on the label, such as name, production and expiry date, as well as a licence number. The law now required the production process to be supervised by a specialist, and it introduced the possibility of official inspections. In addition, the licence had to be renewed once a year, and the product had to be tested for purity and potency.

The law was supplemented by the Food and Drug Act of 1906, which stipulated that the effect promised by the manufacturer on the label had to correspond to what the product could actually accomplish. However, there was still no direct requirement for safety or an efficacious mechanism of action.

One of the more spectacular medical scandals Zindy described involved *Mrs. Winslow's Soothing Syrup* – a widely used product in the last half of the nineteenth century and early twentieth century to cure toddlers' toothaches and diarrhoea. The manufacturer also emphasized the product's ability to effectively quiet restless infants and small children. The product contained both morphine and opium, which explains why it made parents feel that it worked as intended.

The exact number of babies whose death was caused by the product is unknown, but the product often made headlines. The strong depressants in *Mrs. Winslow's Soothing Syrup* dramatically slowed an infant's pulse rate and could induce coma, addiction or death. But people kept using the product because it continued to be sold. As the infant mortality rate was quite high back then, many people never questioned why an infant had died.

An article was published in 1911 which revealed the true ingredients and hazards of





PHOTO: KIM GRANLI

using Mrs. Winslow's Soothing Syrup, but even so the product was not taken off the market until 1930, after having been sold for more than 80 years!

The Elixir Sulfanilamide Tragedy of 1937 caused the death of more than 100 people. Sulfanilamide was the first commercial antibiotic and was marketed to combat streptococcus infections. Unfortunately, in liquid form, the product contained diethylene glycol (DEG). DEG can be used as antifreeze and is now considered toxic. When the case became known to the public, it led to the *Federal Food, Drug and Cosmetics Act of 1938* ("Drug Safety Law"). This law implemented requirements for safety, authorized inspections and prohibited the use of false marketing. The US authorities (the US Food & Drug Administration) could also revoke a marketing permit if it turned out that the product was not safe after all.

One of the more infamous drug tragedies in Denmark – namely the Thalidomide Tragedy, when mothers gave birth to children with

deformities – was also discussed in the presentation, focusing on the tragedy's legislative consequences. As Thalidomide had been developed as an agent to combat morning sickness in pregnant women, the drug had been tested on gestating mice and rats, whose offspring were not affected by deformities. Very high doses of the product were tested on rabbits (25 to 300 times the human dose) and monkeys (10 times the human dose), which caused deformities in their offspring, but as they had been given high doses, the results were deemed insignificant in relation to authorizing the product. The tragedy resulted in a number of legislative reforms to increase drug safety, including in the area of toxicology.

European pharmaceutical legislation began to develop later than in the US but it is now at the same level as in the US.

## THE DANISH 3R CENTER'S 2017 AWARD

The next item on the agenda was the granting of the annual award – the fourth in the history of the centre. The 3R Award went to Grete Østergaard, University of Copenhagen, and was presented by Christine Nellemann.

Christine talked about some of the considerations underlying the choice of Grete Østergaard. Grete Østergaard received her degree in veterinary medicine from the Royal College of Veterinary Medicine and Agriculture in 1983. She later earned a PhD from Roskilde University Centre in 1997 and an MSc in laboratory animal science from the University of Copenhagen in 2006.

Grete worked as a clinical veterinarian from 1983 to 1985, after which she worked as a clinical trials associate for Lundbeck from 1985 to 1988. From 1988 to 2003, she worked as a toxicologist at the Department of Toxicology, under the National Food Institute. In 2003, Grete began working as a laboratory animal veterinarian at the Department of Experimental Medicine, University of Copenhagen, where she is now the senior veterinarian.

In her time at the Department of Experimental Medicine (DEM), Grete has been instrumental in building up the comprehensive Laboratory Animal Care and Use programme at the Faculty of Health and Medical Sciences (SUND), which has resulted in the department being accredited by AAALAC ([aaalac.org/](http://aaalac.org/)).

Grete also helped establish the local animal welfare committee at DEM (<http://emed.ku.dk/om/dyrevelfaerdskomite/>) to ensure that animal experimentation carried out at SUND takes the greatest possible account of animal welfare and the 3Rs. Today, all undertakings where animal experimentation takes place are required by law to have this type of committee, but DEM's animal welfare committee was established long before this was a legal requirement.

Grete's efforts at DEM over the past 14 years have thus been crucial for the immense task of optimizing the welfare of many animals used in research at the University of Copenhagen. Grete is uncompromising in applying her values or in the task of ensuring that rules are complied with.

Every day she tries to optimize animal welfare, which many SUND researchers have certainly observed over the years. In brief, she has been and still is incredibly important to 3R and animal welfare efforts and she is truly a worthy recipient of the 3R Award.

At the conclusion of her speech, Christine Nellemann gave the floor to Grete Østergaard.

Grete Østergaard said that in order to achieve success in the area of animal welfare at a laboratory-animal facility, it is essential to establish a Culture of Care. She told how researchers presumably are most keenly focused on their respective research projects, which is why the animal-facility staff, including veterinarians and animal keepers – who are generally characterized by a deep sense of care for the animals – play an important part in establishing a Culture of Care. Therefore, it is crucial to provide the animal-facility staff with ideal conditions for achieving this.

Grete also cited some of the problems relating to these efforts. She described how animal-facility staff are sometimes unfortunately perceived as intrusive by researchers and how animal-facility staff are not always treated with respect, which can undermine job motivation and ultimately impair animal welfare. Therefore, Grete called for a stronger obligation on the part of researchers to follow the advice of veterinarians and the animal welfare body, such as by adding an amendment to the legislation. After Grete's speech, the symposium audience enthusiastically applauded her great work.



PHOTO: KIM GRANLI

# WINNERS OF THE 3R AWARD

2014 – ELLEN MARGRETHE VESTERGAARD,  
DANISH HEALTH AUTHORITY

2015 – HANNE GAMST-ANDERSEN,  
NOVO NORDISK

2016 – THE QSAR TEAMET, REPRESENTED BY EVA BAY  
WEDEBYE AND NIKOLAI GEORGIEV NIKOLOV,  
TECHNICAL UNIVERSITY OF DENMARK

**2017 – GRETE ØSTERGAARD,  
UNIVERSITY OF COPENHAGEN**

PHOTO: KIM GRANLI



## THE 3R AWARD

Each year, the Danish 3R-Center presents an award to a person or group of persons affiliated with a company, university or other body working to promote the 3Rs in Denmark.

The award is presented as part of the annual symposium. In the period leading up to the symposium, nominations can be sent to the Danish 3R-Center, after which the board determines who should receive the award, which comes with a diploma and DKK 10,000.

**PLEASE NOTE:** You can subscribe to the Danish 3R-Center's newsletter on [3rcenter.dk](http://3rcenter.dk), so you don't miss deadlines for the 3R Award.

# REDUCTION OF ANIMALS USED FOR EXPERIMENTATION THROUGH 3D PRINTING



PHOTO: KIM GRANLI

**David Bue Pedersen**, *Technical University of Denmark*

David Bue Pedersen, Technical University of Denmark (Department of Mechanical Engineering), provided the symposium attendees with insight into a whole other world than the one they normally deal with in their work – namely 3D printing. David is not personally involved in the laboratory animal or alternatives milieu. His talk therefore provided insight into the possibilities of 3D printing, after which he urged symposium participants to contact him with questions about specific problems that could lead to possible cooperation for the benefit of laboratory animals.

David said we should remember that 3D printing technology is still in its infancy, explaining the lack of a broad perspective on its inherent benefits, limitations and drawbacks related to the area of animal experimentation and alternatives.

David presented four 3D-printing methods: *Photopolymerase*, *Material Jetting*, *Powder Bed Fusion* and *Material Extrusion* – to give the participants an idea of the possibilities.

The first-mentioned method involves filling a tub with liquid polymers (which consolidate into a solid material from liquid form) and then using them to manufacture customized hearing aids based on an ear scan. The method can also be used to print hard prostheses for use inside the body, such as skull parts.

*Material Jetting* is comparable to an inkjet printer where things like organs can be printed for doctors to practise on but these can also be printed on a large scale for soft prostheses.

*Powder Bed Fusion* is a method where a laser passes over pulverized metal and hardens it into the preferred shape. This is particularly used in aviation but also for hip implants as it is possible to create a material which tissue can adhere to, thus eliminating the need to use bone cement.

*Material Extrusion* is a method where the material comes out of a small tube and is shaped slowly. It can be used for live cells to print things such as ear cartilage, but it can also be used to make liver and heart tissue. The challenge is to infiltrate it with blood vessels.

The Danish 3R-Center will definitely endeavour to keep up to date on 3D printing going forward, and the centre looks forward to hearing about any future cooperation constellations between the Department of Mechanical Engineering and people involved with laboratory animals or alternatives.

Also, the Danish 3R-Center will certainly consider inviting a researcher to provide an update on the current situation of 3D printing at a future symposium.

# ANIMAL WELFARE AND THE 3RS AT ROCHE

**Tobias Schnitzer**, *Roche*

The final international speaker was Tobias Schnitzer from the pharmaceutical company Roche. Due to its very size, Roche uses many laboratory animals in a global perspective, although this number has been almost cut in half in recent years from roughly 550,000 in 2010 to around 300,000 in 2016 (by comparison around 255,000 laboratory animals were used in Denmark in 2016 (latest figure)). Roche endeavours to take due account of animal welfare and to develop alternatives, which it does by using and exploring alternative methods, applying high global standards, etc.

Tobias described how Roche is working to develop many types of alternatives, such as human tissue, computer simulation, organs-on-a-chip, but also new animal models as an alternative to existing animal models, such as minipigs as an alternative to non-human primates.

In the long term, the corporation has great expectations of organs-on-a-chip, as researchers have developed three-dimensional (3D) organ-like tissue models, which are particularly capable of contributing to drug development in the early phases, i.e. in the selection of drug candidates, and thus minimize the use of animals. (See also the article *The application of 3D cell models to support drug safety assessment: Opportunities & Challenges* (Adrian Roth and Thomas Singer).)

Roche also focuses on the possibilities provided by zebra fish in areas such as safety pharmacology and toxicology, where the corporation now has a fully operational zebra fish facility. Roche has successfully bred and raised zebra fish larvae and they continuously evaluate zebra fish as a model in relation to the above-mentioned branches of science. Roche also maintains unwavering focus on possible refinement initiatives relating to the zebra-fish facility.

Roche grants a 3R award every two years to recognize and promote 3R innovation in the company. All employees are eligible for the award, including animal keepers, researchers and management. The number of applications for the award has been steadily rising since the first award was presented in 2008: from 13 applications in 2008 to 67 in 2017, indicating that Roche has successfully promoted the 3R mindset in the corporation.

Tobias Schnitzer also described how the corporation supports institutions that promote and develop alternatives (replacement), from the future Swiss National Centre of Competence for the 3Rs (successor to the 3R Research Foundation Switzerland), Center for Alternatives to Animal Testing Europe (CAAT EU) and the European Partnership of Alternatives to Animal Testing (EPAA) and many others. Roche also focuses on external 3R collaboration with various national and international organizations, specifically mentioning NC3Rs, the European Federation of Pharmaceutical Industries and Associations (EFPIA) and SC4SM (Stem Cells for Safer Medicines).

It is also important to Roche to apply and implement high standards in its work and these must be more stringent than required by law, exemplified by animal-welfare inspections of internal and external facilities alike conducted by Roche's own veterinarians. Roche is also accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International), a private, non-profit organization working to promote humane treatment of animals within science through voluntary accreditation and assessment programmes.

Tobias Schnitzer concluded his remarks by describing the corporation's efforts targeting PR, dialogue and transparency, exemplified by allowing students, politicians, etc., to visit its facilities and by Roche's willingness to assist researchers and students with questions/theses relating to animal experimentation.



## ANIMAL PAIN – THE ENEMY OF SCIENCE, WELFARE AND ETHICS



PHOTO: KIM GRANLI

**Carsten Grøndahl**, *Chief Veterinarian, Copenhagen Zoo*

Carsten Grøndahl has left his mark on the development of veterinary work particularly in the area of exotic animals, specifically animal anaesthesia. And pain and anaesthesia were the very topics of Carsten's talk at the annual symposium.

Carsten Grøndahl started by stating that our ability to effectively treat pain in animals requires us to be able to measure their pain, which is why a large-scale effort is being made at global level to increase the understanding of and attentiveness to pain in animals.

Carsten explained how the nature of pain in animals can be even more complex than in humans, and our knowledge of how animals actually experience pain is still inadequate. How an animal experiences pain and how it expresses this pain are not quite comparable to how we experience and express pain. For good reason, animals cannot verbalize their pain. It's true that a dog can bark, but what about rabbits, horses or tortoises? Therefore, animals depend on observations (by humans) to recognize and assess the severity and impact of their pain. Animals' inadequate ability to understand calming words and explanations, such as in the lead up to surgical intervention, about how the intervention will relieve the pain from a broken limb, for instance, could mean that animals actually suffer more than we humans do.

Carsten Grøndahl illustrated the background for the perception of pain in animals. He touched on how challenging it can be to

assess the subjective effect of pain on an animal. To address this, he presented a number of behavioural indicators that can be used to identify an animal in pain.

The presentation continued by thoroughly reviewing the characteristics of pain. The different types of pain are differentiated according to degree, duration, location and source. This means that the specific pain therapy must be adapted to the type of pain involved to provide the best possible relief. For minor pain, a mild analgesic of just one drug will do, such as NSAIDs (non-steroidal anti-inflammatory drugs). More intense pain requires multimodal intervention, i.e. pain relief involving more than one drug.

Ideal pain relief helps ensure animal welfare by suppressing both physical and mental distress during pain-inducing procedures. But it is also essential for ensuring ideal healing in the long term. Pain relief also has a preventive effect by inhibiting consequential effects, such as phantom pain and the accumulation of stress hormones, which can adversely affect research results.

On the other hand, the fact that analgesics can have inexpedient side effects must also be considered. Therefore, it is important to treat pain in moderation and select the gentlest alternative. Similarly, it is important to plan the pain management programme so it fits into the experiment schedule and that the pain relief lasts long enough between treatments.

In the immediate future, these efforts will rely on the development of combined and improved analgesics. New ways to administer the drugs, such as slow-release gels and long-acting drugs, must also help optimize treatments. In addition, research is looking at the significance of genes to the analgesic

effect and long-term treatment of chronic pain. In a slightly longer-term perspective, it is also hoped that it will be possible to fine-tune drugs and find new methods so pain relief can be even more targeted and its side effects minimized.

## CLOSING REMARKS

**Adrian Smith**, *the Danish 3R-Center*

Adrian Smith ended two rewarding days with his closing remarks where he urged researchers and others to publish their 3R improvements to heighten awareness of them and disseminate them in laboratory animal circles – a plea that is well in keeping with Adrian's work at Norecopa and the Danish 3R-Center, as both are keenly focused on communication.

Adrian concluded by thanking participants and speakers and expressing his hope of seeing everyone again at next year's symposium in 2018.

### THE DANISH 3R-CENTER'S 2018-SYMPOSIUM

will be held on 5–6 November at Charlottenhaven in Copenhagen. Sign up for the Danish 3R-Center's newsletter at **3rcenter.dk** so you don't miss the registration deadline, etc.

## SATISFACTION SURVEY ABOUT THE SYMPOSIUM

After having conducted a satisfaction survey among symposium participants, we were able to conclude that the 2017 symposium was the most successful ever held by the Danish 3R-Center.

Sixty participants took time to respond to the questionnaire, where each participant was asked to rate his/her level of satisfaction (or dissatisfaction) on a scale of 1 (dissatisfied) to 5 (very satisfied), which resulted in an average rating of no less than 4.6.

During the planning of the symposium programme, the Danish 3R-Center chose to focus not only on presenting an interesting topic but also on ensuring that the topic was presented by a good communicator. According to the survey results, it appears that this approach was rewarded and is worth using in preparing for the 2018 symposium.

# OPENING OF THE SWEDISH 3RS CENTER 21 NOVEMBER

The Danish 3R-Center was represented at the official opening of the Swedish 3Rs Center. The Danish 3R-Center has long awaited the launch of a Swedish counterpart as it is expected to lead to a rewarding partnership.

The official opening was held at the Swedish Board of Agriculture in Jönköping and was attended by around sixty people. It started with a speech by Leif Denneberg (Director General of the Swedish Board of Agriculture), where he notably emphasized the importance of international cooperation:

*“The centre must coordinate the efforts of various players at animal experimentation undertakings and encourage an environment where we learn from one another – internationally and nationally. This will make it easier to work on alternative methods to animal experimentation. Together we will cultivate our knowledge and let it be permeated by the 3R principles.”*

The Danish 3R-Center was not just a guest at the opening but it was also represented on the programme, as board members Lisbeth E. Knudsen and Adrian Smith gave talks on *Replacement and Refinement* respectively.

As this was the opening of a 3R centre, there were presentations on each of the 3Rs: Replacement, Reduction and Refinement, as the 3R concept was not necessarily familiar to all participants. The “reduction” presentation was given by Maria Karlgren of Uppsala University, who described the methods she uses in her research to reduce the number of laboratory animals.

Lisbeth E. Knudsen described the current situation in the area of alternative methods (replacement). The development of alternatives to laboratory animals have been in focus over the past fifty years, based on toxicology. The European Union established the research institution *European*

*Committee on the Validation of Alternative Methods* (ECVAM) with this objective more than 25 years ago. Many research projects are initiated by the EU and have subsequently become part of international initiatives.

Initially, in vitro toxicological testing was the object of focus, e.g. cell-based testing to determine vaccine purity. Since then, in silico methods have been added; these are computer based and use accessible data on related substances as their point of departure. Lisbeth also dealt with *Adverse Outcome Pathways*, which combine molecular knowledge with knowledge of outcomes.

At present, the use of alternatives is primarily driven by the European Directive on the protection of animals used for scientific purposes. Lisbeth presented the results from the project initiated by the Danish 3R-Center on the familiarity of animal experimentation practitioners with the 3Rs and emphasized the importance of finding good examples for compliance and training.

Adrian talked about animal welfare and scientific benefits provided by refinement, which is why Adrian presented Norecopa’s PREPARE guidelines for the planning of procedures involving laboratory animals (*Planning Research and Experimental Procedures on Animals: Recommendations for Excellence*). The guidelines have so far been translated into thirteen languages and can be found on Norecopa’s website or at [3rcenter.dk/forskning/forbedr-din-forskning](http://3rcenter.dk/forskning/forbedr-din-forskning).

The opening event was deemed rewarding by the Danish 3R-Center, especially because of an expressed wish for cooperation by a number of Swedish representatives. Josefine Zidar, one of the Swedish 3Rs Center eight employees, put it this way: *“We have a lot of inspiration and knowledge to obtain from our neighbouring countries.”*

# EUROPEAN PARTNERSHIP FOR **ALTERNATIVE APPROACHES TO ANIMAL TESTING** NOVEMBER

*The Partnership for Alternative Approaches to Animal European Testing (EPAA) held its annual conference in Brussels, attended by almost 80 participants. The topic was the building up of synergies for the purpose of accelerating the development and acceptance of alternative methods to animal experimentation.*

The speakers included representatives from the European Parliament, *the Federation for Laboratory Animal Science Associations (FELASA)* and *the European Federation of Pharmaceutical Industries and Associations (EFPIA)*.

The annual refinement award, given to paraveterinary workers or animal keepers, was presented during a ceremony at the conference. The award went to Camilla Bengtsson and Marie Eriksson of Swetox in Sweden for their study of how the handling and training of mice and rats result in calmer animals during experimental procedures. The conference rounded off with a panel debate with representatives from, among others, *the National Centre of the Replacement, Reduction & Refinement of Animals in Research (NC3Rs)*, *the European Food Safety Authority (EFSA)* and the European Commission. The panel debated knowledge-sharing between different sectors, and a possible need for new forums was discussed.



PHOTO: CHRISTINA HAUSCHILDT



# 4

PHOTO: NOVO NORDISK



## 3R ACTIVITIES IN THE RESEARCH GROUP FOR MOLECULAR AND REPRODUCTIVE TOXICOLOGY AT THE NATIONAL FOOD INSTITUTE

Over the past two years, we have asked Aarhus University and the 3R department at Novo Nordisk respectively to describe their 3R efforts for our annual reports. This year, we asked the research group from Molecular and Reproductive Toxicology at the National Food Institute to describe their efforts to integrate the 3Rs into the use of computer, cellular and animal models. We would be happy to hear from other institutions that would like to describe their approach to making a difference in the 3R area.

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***By Eva Bay Wedebye, Marianne Dybdahl,  
Sofie Christiansen and Terje Svingen***

Traditionally, animal experimentation has been crucial in chemical assessments, and some questions can still only be answered by means of animal experimentation. In our group at the National Food Institute, we conduct research into, among other things, the development of animal-free methods to help achieve a paradigm change in how risk assessments of chemicals can be done in the future.

Molecular and Reproductive Technology is a large multidisciplinary research group at the National Food Institute. Our overarching aim is to protect humans against possible harmful effects that can occur when they are exposed to environmental chemicals. We have a long-standing tradition of examining especially potential hazardous effects that arise when a foetus is exposed to chemicals with endocrine-disruptive effects.

In order to chart the specific effects of various substances and advise authorities and others how best to protect people against these same effects, we apply different strategies which jointly help us to achieve these goals in the best possible way. We take a holistic approach and use computer modelling, cell-based methods and animal experimentation to illustrate the problem. However, we only carry out animal experimentation where this is necessary, and we continuously endeavour to base our research and advise on the 3R principles. Thus, the group works to find alternatives to animal experimentation (Replacement), to develop methods which use as few animals as possible and ensure that we get the most out of the laboratory animals that still have to be used (Reduction) and to ensure that the animals are provided with the best possible conditions (Refinement).

### **In silico approach**

Over two decades, our research group has developed many Quantitative Structure-

Activity Relationship (QSAR) computer models to predict the harmful impact of chemicals based on the chemical structure. We made our database of QSAR predictions freely available on the internet ([qsar.food.dtu.dk](http://qsar.food.dtu.dk)) in 2015. The models build on previous results from human, animal and cell models.

We use QSAR predictions to assess chemicals, usually together with information from other alternative methods or with historical data from animal experimentation whose quality is not sufficient to stand alone. The model predictions can be used to set priorities to bring focus to bear on the most problematic chemicals, and they can help in the design of safer chemicals that have a lower risk of subsequently turning out to be problematic and prompt comprehensive animal experimentation.

In addition, the research group develops and uses other in silico methods, such as physiologically-based kinetic (PBK) modelling. PBK models enable us to predict the fate of chemical substances in the body, i.e. absorption, distribution, conversion and excretion. These items are integrated into the animal model and animal-experimentation procedures, and they are also important to identify in an alternative approach based on cell-based procedures and so-called non-test methods.

### **In vitro approach**

Another important area of the group's research is conducted using cell-based (in vitro) testing methods. These are primarily based on human cells, but in some instances on animal cells as well. These cell-based testing methods are often particularly well-suited for determining how chemical substances affect the body at molecular level and thus can give rise to undesirable effects. As chemical substances can affect humans in

many different ways, the group has focused on building up a wide spectrum of in vitro testing methods which jointly cover many different mechanisms.

Seeing that procedures based on human cells are expected to be best suited for predicting effects notably in humans, much of the group's research involves the development of additional new in-vitro methods based on such cells, e.g. human stem cells. We use in vitro models particularly in our research into endocrine-disruptive effects and, in this context, we have established a panel of methods for studying the efficacy on sex steroids, thyroid hormones, etc. We are continuously enlarging the panel of methods with a view to identifying additional relevant mechanisms. Other approaches we use in the group to illuminate underlying mechanisms are so-called omic and high-content technologies.

### **In vivo approach**

When computer calculations and in vitro testing in themselves do not provide enough knowledge to be able to protect the population against possible harmful effects from environmental chemicals, we at the National Food Institute have animal experimentation facilities that are set up to be able to conduct animal experimentation. These procedures can provide information on hazardous or beneficial effects of dietary factors, chemical substances and products, as well as micro-organisms, including genetically modified micro-organisms. Our group conducts reproductive procedures in gestating animals and examines the impact of different chemicals on offspring. The advantage of in vivo experiments is that they make it possible to measure the effect on the entire organism or the developing foetus.

Many different animal-experimentation methods are used to study whether chemical substances can affect foetuses and offspring. Many of these methods are standardized and, for example, the OECD has developed



guidelines (recommended methodological precepts) describing how to use the methods. The methods are used for regulatory testing of chemical substances.

### **Overarching perspective**

Developments in the area of molecular biology and toxicology will in future pave the way for developing better alternative methods for assessing chemicals. The use of traditional animal experimentation for assessing the hazard of chemicals will gradually be replaced or supplemented by the use of alternative methods such as in vitro examinations and computer-based predictions. It is also expected that more targeted animal experiments will be eventually be used in instances where they are indispensable. This transformation is already under way, and we actively contribute to these efforts, including together with the Danish EPA under the auspices of the OECD and EU.

### **Contributing to international work**

Our research group actively participates in OECD efforts to develop Adverse Outcome Pathways (AOP) and Integrated Approaches to Testing and Assessment (IATA). For each harmful effect, an AOP systematically describes the available knowledge of the biological mechanisms causing the effect. Charting correlations and basic mechanisms makes it possible to use relevant mechanical knowledge from things like cell-based methods and QSAR to predict harmful effects in humans. AOPs covering a wide range of different effects in areas such as cancer and damage to the reproductive and nervous systems are freely available at the AOP wiki website ([aopwiki.org/](http://aopwiki.org/)).



IATA combines all available information from in silico, in vitro and in vivo studies relating to a chemical assessment. The combined use of these methods can reinforce the interpretation of individual data, which is why IATA could prompt authorities to increasingly use data from alternative methods. IATA can also guide a test strategy where new experimental investigations are required. Together with the Danish EPA, we contribute to the OECD's efforts, including in relation to the development of guidelines on the use of IATA aimed at ensuring that the authorities use mechanistic knowledge in a uniform manner.

The OECD Test Guideline Programme (TGP) develops internationally recognized standard testing methods that are used pursuant to the Danish Chemicals Act. Since 2010, our group has been one of two national (Danish) coordinators of the OECD Test Guideline Programme. When a test is carried out using

an OECD Test Method, the Member State mutually accepts the data generated in the test. This means that companies do not need to test their substances for the same effect in different procedural designs, depending on where in the world the substances are used. A number of in vitro methods have been developed as OECD Test Guidelines, and our research group actively participates in these efforts. It often takes several years to validate, standardize, comment on and adopt these methods.

Our research group has also been very active in efforts to develop new and improve existing in vivo reproduction guidelines with different endocrine-relevant end points. By enlarging the test methods, they can now be used to a much greater extent than previously to assess the possible endocrine-disruptive effects of chemicals. This improvement (Reduction) will be carried out without using more animals than those already in the procedure.

## QSAR TOOLBOX + DATABASE

For many years, we have been working to increase the use of QSAR and other animal-free methods in international assessments of chemicals in the EU and OECD. We do this together with the Danish EPA by demonstrating how the methods can be used when we, for example, contribute to assessments of specific substances or to prioritization exercises, and we also

contribute to international guidelines in the area. At the OECD, we have been an active team player in terms of authorities giving higher priority to the use of animal-free methods, and we contribute to efforts to develop the OECD's and EU's QSAR Toolbox which everyone is free to use for their alternative assessments of chemicals.





# 5

PHOTO: THE DANISH 3R-CENTER

## INTERNATIONAL COOPERATION

In 2017, the Danish 3R-Center continued to focus on international cooperation, as the relatively modest size of our centre makes such cooperation logical in our efforts to disseminate information about the 3Rs. In addition to our vast networking tasks, taking place at the previously described meetings and conferences in which the Danish 3R-Center takes part, more specific collaborative efforts are ongoing or in the pipeline: the first-mentioned relates to Norway's Norecopa and the last-mentioned relates to the newly-established Swedish 3Rs Center.

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# NORECOPA

The Danish 3R-Center feels a special bond with Norecopa, as Adrian Smith is both secretary for Norecopa and board member for the Danish 3R-Center. In recent years, this has resulted in promotional efforts where both centres promote one another's events and activities, share relevant news and keep one another informed of ongoing efforts.

Norecopa has developed guidelines for planning animal experimentation, which complement the NC3Rs' ARRIVE guidelines, as Norecopa's guidelines also address the issue of planning before conducting the animal experiments (PREPARE stands for Planning Research and Experimental Procedures on Animals: Recommendations for Excellence).

The purpose of PREPARE is to help researchers improve the quality of their animal experimentation and to improve animal welfare at the same time. PREPARE also encompasses the health-related, environmental and safety aspects of the procedures.

PREPARE includes a checklist, advice on cooperation between researchers and the animal facility, as well as websites which explain the checklist in more detail. The websites also include links to specific international guidelines for all topics on the checklist.

# PREPARE

[norecopa.no/prepare/prepare-checklist](http://norecopa.no/prepare/prepare-checklist)

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## DESIGN OF A STUDY

### 1. INFORMATION RETRIEVAL

- Articulate a clear hypothesis, with primary and secondary objectives.
- Consider carrying out a systematic review of the literature available (Systematic Review).
- Decide which databases and information specialists to use and devise search words.
- Assess the relevance of the species to be used, its biology and suitability for answering the experimental/scientific questions with minimum suffering and meeting the species' welfare needs.
- Evaluate the project's reproducibility and whether it can be applied to other animal species.

### 2. LEGAL QUESTIONS

- Assess how the procedure is covered by relevant legislation for animal experimentation and other relevant areas, such as animal transport and occupational health and safety.
- Search for relevant guides and guidelines (e.g. EU project evaluation guidelines).

### 3. ETHICAL ISSUES, FULFILMENT OF THE BENEFIT CRITERION AND HUMAN END POINT

- Write a summary of the project in layman's language.
- Assess, in dialogue with animal-welfare bodies, whether statements on this type of procedure have already been published.
- Consider the "3Rs" (Replacement, Reduction, Refinement) and the "3Ss" (Good Science, Good Sense, Good Sensibilities).
- Consider registering the procedure in advance and publishing negative results.
- Analyse the severity in relation to benefits/utility value and justify the necessity of any suffering that might occur during the procedure.
- Discuss the learning targets if the animals are to be used for teaching or training purposes.
- Classify the project by severity.
- Define objective, easily quantifiable and unequivocal human end points.
- State the reasons for needing to use death (if this need exists) as an end point for the procedure. (Death as an end point is illegal in Denmark.)

### 4. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

- Assess the necessity of a pilot experiment, statistical power and level of significance.
- Define the experimental unit and determine the number of laboratory animals to be used.
- Decide on methods for randomization, avoid observational bias and determine the inclusion and exclusion criteria.

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## DIALOGUE BETWEEN RESEARCHERS AND THE ANIMAL DEPARTMENT

### 5. TARGETS AND TIME SEQUENCE, FINANCING AND DISTRIBUTION OF WORK

- Organize meetings with all relevant staff groups once the early plans for the project exist.
- Present an approximate time line for the project showing the need for assistance with preparations, accommodation and care, procedures and waste management/decontamination.
- Discuss and highlight all expected and potential costs.
- Present a detailed plan showing the distribution of tasks and expenses at all stages of the experiment.

### 6. EVALUATION OF THE ANIMAL UNIT

- Physically inspect the facilities to assess the condition of buildings and equipment and to determine any special needs.
- Discuss the need for staffing during periods of heightened risk.

## 7. EDUCATION AND TRAINING

- Assess the existing skill-sets of the staff and, if necessary, revise the need for supplementary training and courses before the procedure.

## 8. HEALTH ASSESSMENT AND OHS, WASTE MANAGEMENT AND DECONTAMINATION

- Assess the risks together with the animal unit, comprising all individuals and animals directly or indirectly affected by the study.
- Assess and, if necessary, draw up specific guidelines for all stages of the project.
- Assess methods for verifying and safeguarding infectious agents and other substances posing a biological risk, for the decontamination of all equipment that will be used in the study and the disposal of waste.

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# QUALITY CONTROL OF THE VARIOUS PARTS OF THE STUDY

## 9. TEST SUBSTANCES AND TESTING PROCEDURES

- Provide as much information as possible about the test substances.
- Evaluate the feasibility and scientific suitability of the test procedures and the practical skills required to carry them out.

## 10. LABORATORY ANIMALS

- Determine which essential characteristics the animals must possess for the study and subsequent reporting.
- Avoid producing too many surplus animals.

## 11. QUARANTINE AND HEALTH MONITORING

- Discuss the animals' probable health status and any needs they might have for transportation, quarantine and isolation, as well as health monitoring and consequences for the staff.

## 12. ACCOMMODATION AND CARE

- Consider the animals' specific instincts and needs by consulting experts.
- Discuss acclimatization, ideal housing conditions and procedures, environmental factors and any restrictions on these (e.g. fixed or single housing).



### 13. EXPERIMENTAL PROCEDURES

- Optimize and adapt methods for capture, immobilization, labelling and setting free or rehoming.
- Optimize and adapt methods for treating the animals and for taking samples, administering sedation and anaesthesia and performing surgery and other interventions.

### 14. HUMANE KILLING, SETTING FREE OR REHOMING

- Search for relevant legislation and guidelines well in advance of the study.
- Define the primary methods for killing and methods that can be used in an emergency situation.
- Assess the skill-sets of the person who will be carrying out these tasks.

### 15. AUTOPSY

- Prepare a systematic plan for all stages of the autopsy, including where it should take place and identifying all the animals and samples to be taken.

The Danish 3R-Center supports Norecopa in the dissemination of PREPARE, such as here in this report and on the website [3rcenter.dk/forskning/forbedr-din-forskning](http://3rcenter.dk/forskning/forbedr-din-forskning)

## THE SWEDISH 3RS CENTER

The Swedish 3Rs Center is mentioned several times in this annual report, which is due to both centres' wish for future cooperation.

Initially, the Danish 3R-Center envisions cooperation that is comparable to the cooperation with Norecopa where the two centres can help one another in promoting events, research results, relevant news, etc.

The Swedish 3Rs Center is still in a start-up phase, so it is still not completely clear how cooperation will manifest itself in detail (the rules of procedure for the National Committee to which the Swedish 3Rs Center is affiliated

were issued on 21 December 2017 and came into effect on 1 January 2018.

The Danish 3R-Center has held several informal meetings with Torsten Jakobsson, the Project Manager of the Swedish 3Rs Center, and Mats Sjöquist, who is a member of Sweden's National Committee. At these meetings, everyone agreed that cooperation should be established. From the Danish point of view, we feel certain that both centres can benefit greatly from the above-mentioned promotional cooperation, but it could definitely be interesting to cooperate on bigger projects.



PHOTO: THE DANISH 3R-CENTER

# APPENDIX

## The History of the Danish 3R-Center

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As early as 2005, the Danish government decided to establish the Danish Consensus Platform for Alternatives to Animal Experiments (DACOPA), under the European network the European Consensus Platform for Alternatives (ecopa).

The purpose of DACOPA was to bring together representatives of animal protection organizations, private-public research and authorities to seek consensus on animal testing issues with a particular view to promoting the 3Rs.

DACOPA consisted of a chairman and two representatives of each of the four above groups which provided an opportunity to share lessons learned and discuss how to promote the 3Rs in Denmark and abroad in the best possible way. However, DACOPA was challenged by a lack of funding for launching research projects and there was no secretariat assistance for performing the tasks that were desired implemented.

This was not satisfactory for the groups of stakeholders, which was expressed in a stakeholder analysis carried out in 2011/2012 among all interested parties in the

field of laboratory animals. Almost unanimously, the feedback was that Denmark should either set up a 3R-Center inspired by the Britain's *National Centre for the Replacement, Refinement & Reduction of Animals in Research* (NC3Rs) and Germany's Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch am BfR ZEBET) or allocate sufficient funding to DACOPA.

Following negotiations between the Ministry of Food, the pharmaceutical industry and a number of animal welfare organizations, it was agreed in the spring of 2013 to establish the Danish 3R-Center with a scientific board, a budget of its own, research funding and a secretariat. The Alternative Fund, the Danish Animal Welfare Society, the Danish Laboratory Animal Protection Society, LEO Pharma, Lundbeck and Novo Nordisk decided to contribute funding for the project, and the Danish Ministry of Food offered operational and research funding (the Alternative Fund and the Danish Animal Welfare Society are no longer contributors to the Danish 3R-Center). The Cooperation Organization of Animal Welfare Organizations (DOSO) is a contributor of the Danish 3R-Center from and including 2017.

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# THE 3RS

## REPLACEMENT, REDUCTION AND REFINEMENT

William Russell and Rex Burch are behind the concept of the 3Rs which they described in the scientific study *The Principles of Humane Experimental Technique* in 1959.

The increasing use of laboratory animals in research made it necessary to focus on animal welfare and the ethical issues involved in subjecting them to pain and related stress.

The wish to enable the research community to collectively follow certain guidelines to reduce the number of laboratory animals and alleviate animal suffering became Russell and Burch's 3R concept – *Replacement, Reduction and Refinement*. The following are the definitions of the 3Rs which the board of the Danish 3R-Center has developed further based on a definition articulated by DACOPA, with inspiration from Russell and Burch.

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### REPLACEMENT

Replacement means that experiments involving live animals (animals comprised by the Danish Animal Protection Act) are replaced with experiments that do not use whole, living vertebrates. Replacement could thus be in the instances where experiments are made on 1) cells or isolated organs, 2) dead vertebrates, 3) invertebrates (except for cephalopods), plants or micro-organisms, 4) synthetic or electronic materials and 5) human volunteers.

### REDUCTION

Reduction of the number of animals used to obtain a certain amount of knowledge with the requisite precision.

Reduction comprises instances where a smaller number of animals can be used in a given situation in a specific experimental model than previously. Consequently, reduction must always be a target for lowering the number of animals used for generating a certain amount of knowledge and not as a target for whether a given organization, state or company has reduced the overall number of animals within a given time frame compared to corresponding previous periods of time.



The development of animal models with a view to enhancing the scientific results achievable from each individual animal is therefore also considered reduction. Reduction can be obtained using measures such as screening with animal-free models or technologies prior to animal testing, by using animals with the exact characteristics that one is interested in or by designing more systematic experiments.

## REFINEMENT

Any reduction in the incidence or severity of distress inflicted on the animals that are still necessary to use. Refinement represents the instances where the distress experienced by the individual animal from being used in a specific type of experiment is less than for previous runs of the same type of experiment. This welfare improvement can be achieved both by improving the procedures used as part of the experimental set-up or by improving the framework applying to the animal.

As is apparent in the definition of refinement, it overlaps with the principle of reduction, but refinement is directed more at actual experimental practice than the number of animals relative to the amount of knowledge. Existing methods can be refined with a view to increasing quality of life, for instance through pain relief or improved animal facilities that cater for the natural needs of the different species.

Another significant area of focus for the principle of refinement is the so-called humane endpoints that concern criteria for killing, aborting an experiment or pain treatment. Often, refinement can consist of an endpoint being defined as early symptoms of toxic poisoning or disease rather than letting the toxic poisoning or the disease play out.





## RESEARCH PROJECTS SUPPORTED IN 2016

PHOTO: NOVO NORDISK

In 2016, the Danish 3R-Center received fourteen applications for grants, three of which received a total of DKK 1.5 million.

### TOWARDS BETTER TREATMENT OF **BRAIN CANCER** WITH NEW CELL-BASED MODELS AND LESS ANIMAL EXPERIMENTATION

#### COMPLETED PROJECT

**Bjarne Winther Kristensen**, *University of Southern Denmark*

The purpose of the project was to investigate whether two new cell-based models could replace animal experimentation. In the first model, we inserted cancer cells from humans into slivers of brain tissue taken from mice.

The results showed that the cancer cells quickly spread to the brain tissue in the same manner as seen in patients. In addition, the results showed that the cancer cells expressed stem cell markers.

In the other model, we looked at whether cancer cells could spread in a flat life-like manner in a stem cell culture medium. The results showed that the cancer cells were highly capable of spreading, just as the expressed stem cell markers had a cancer stem-cell phenotype. Experiments with drug candidates showed that the model using brain

tissue was the best predictor of the efficacy of the drug candidate, compared to previously performed animal studies.

We conclude that we have established two new cell-based models where cancer cells spread as in patients and retain their cancer stem-cell characteristics at the same time. It appears to be important that the cancer cells spread in brain tissue in order to be able to predict the efficacy of some drugs. The model in which cancer cells spread in brain tissue provides an opportunity for less costly and faster procedures with new cancer medicine and it thus has the potential to benefit both animal welfare and cancer therapy.

#### ARTICLE

The first set of results from the project was recently published in *Journal of Neuro-Oncology* (2016, 130(1):53–62) and *PLOS ONE* (2016, 11(7) e159746).

## DEVELOPMENT OF COMPUTER MODELS FOR PREDICTING CHEMICALS' IMPACT ON **THYROID HORMONES**

### COMPLETED PROJECT

**Marianne Dybdahl**, *Technical University of Denmark*

Chemicals that might interfere with the body's endocrine systems, causing detrimental effects, have become a source of increasing worry. Up until now, the keenest focus has been on chemicals that affect the balance of sex steroids, but the effects on thyroid hormones have now been brought front and centre in the spotlight as well. In addition to the part they play in the metabolism, the thyroid hormones also play an important part in early brain development. Even moderate and transient reductions of the mother's hormone levels during pregnancy can adversely affect the development of the child's brain and nervous system.

Chemicals can affect thyroid hormones in a wide variety of ways, such as by altering the production of hormones or changing their conveyance through and conversion in the body. For this reason, it is important to use a battery of different testing methods to examine the possible effects, which is a resource-intensive task for the many thousands of untested substances.

To facilitate this task, computer modelling has become an important tool for screening and prioritizing chemicals for further testing. This makes it possible to reduce costs and minimize the number of laboratory animals. The National Food Institute uses computer models to predict the harmful effects of chemicals based on their molecular structure. The models are so-called quantitative structure-activity relationships (QSARs).

This project aims to develop computer models for some of the many mechanisms by means of which chemicals can affect thyroid hormones. Among the methods to be used by the research group to develop the models are experimental test data to which they have been given access through collaboration with the US Environmental Protection Agency.

The new models will be used to screen more than 600,000 chemicals, including the some 70,000 chemicals currently on the European market.

The predictions will be made available in a free and public online database. A virtual screening of this nature can identify potential endocrine-disruptive substances among the many chemicals in our diet, environment and consumer products quickly and inexpensively. The new models can also contribute to the future design of safer drugs and chemicals.

### Results

A number of QSAR models covering different mechanisms have been developed and validated. The models can predict whether chemicals can affect the synthesis and metabolism of the hormones. The new models have been used to screen around 70,000 REACH substances, i.e. substances which are potentially found on the European market.

In so doing, the knowledge from experimental data about a limited number of chemicals was used to generate information for tens of thousands of untested chemicals.

The predictions will be made freely available on the National Food Institute's QSAR database.

The models can identify potential endocrine-disruptive substances among the many chemicals in our diet, environment and consumer products quickly and inexpensively. The new models can also contribute to the future design of safer drugs and chemicals.

The results are also included in a PhD report: [http://orbit.dtu.dk/files/137601994/PhD\\_afhandling\\_Sine\\_Rosenberg\\_print.pdf](http://orbit.dtu.dk/files/137601994/PhD_afhandling_Sine_Rosenberg_print.pdf)

### ARTICLES

- Computational Toxicology 2017; 1:39-48
- Computational Toxicology 2017; 4:11-21
- QSAR development and profiling of 72,524 REACH substances for PXR activation and CYP3A4 induction (Science Direct)
- QSAR models for thyroperoxidase inhibition and screening of U.S. and EU chemical inventories (Science Direct)



PHOTO: CHRISTINA HAUSCHILDT

## USING **CELL CULTIVATION TECHNIQUES** TO MINIMIZE THE NEED FOR LABORATORY ANIMALS IN THE DEVELOPMENT OF VACCINES FOR FARMED FISH

ONGOING PROJECT

**Niels Lorenzen**

It is better for both humans and livestock to prevent infectious disease rather than treating it after the disease has emerged. This is also true of fish farming where a heightened use of vaccines over the past 30 years has led to a sharp decline in the use of antibiotics. The development of vaccines for fish and quality checks of manufactured vaccines is largely based on vaccination and infection procedures involving laboratory animals.

This project aims to develop protocols and methods for using cell cultures in the initial studies of how vaccine components are absorbed in and affect live cells, including also a toxic effect. The work will include both the analysing of gene regulation and microscopic examination. The project will be associated with other ongoing research projects involving vaccination testing on fish, and consequently the project will not use any additional laboratory animals.

The results are expected to contribute to replacing at least some of the laboratory animals used with cell cultures in the initial stages of the development and production of fish vaccines. This will help reduce the need for laboratory animals in vaccine research and

industry and refine animal testing by reducing the level of distress as components with toxic side effects can be sorted out before being tested on animals.

### **Project status per December 2017**

Aquaculture is a rapidly growing food industry which is increasingly replacing fisheries of wild fish populations as a supplier of nutritious animal ingredients for human consumption. Like other types of livestock breeding, loss of animals due to outbreaks of disease and the need for treatment with antibiotics is a big challenge for aquaculture. Prophylactic treatment is better than cure and substantial research has been invested in the development of efficacious vaccines for farmed fish. Successful vaccines against major bacterial diseases have been helping to reduce the need for antibiotics in salmon fish farms in Norway by a factor of 10 since 1987.

In Denmark, we endeavour to achieve similar results by developing more and better vaccines for aquaculture fish. The development of good vaccines is largely based on vaccination experiments under laboratory conditions and means, just like potency-tests of already

commercialized vaccines, that a relatively high number of fish must be used for experimental trials both in Denmark and other countries.

The goal of this project has been to study whether fish-cell cultures could replace the use of live animals to some extent for studies of the effects of vaccine components. Vaccines must be able to trigger both the innate and adaptive components of an immune system, and the project group have used a genetically modified variant of an infectious fish virus which carries the gene for green fluorescent protein (GFP) in studies of the virus's ability to evade both types of immune system mechanisms in a cell culture. Time-course studies of cell cultures expressing interferon after inoculation with the potential vaccine adjuvant poly (I:C) showed that, whereas the cells stepped up their expression of key immune-response mechanisms and were resistant to the viral infection shortly after stimulation, the effect was only temporary and

an extended incubation allowed a latent viral infection to continue and spread to all cells.

The inoculation of receptive cell cultures with a virus in the presence of a powerfully neutralizing antibody showed that the virus within a single passage was capable of evading the neutralizing effect by means of a point mutation in the viral cell surface protein.

The results show how cell cultures can be particularly useful in studies of how vaccine-induced immune responses can interfere with a viral infection and that it is important to know the limitations of these mechanisms when designing a vaccine.

We plan to publish the results in a scientific journal and thus help heighten awareness of the potential for replacing some laboratory fish with cell cultures in efforts to develop and test vaccines for aquaculture fish.

## PREVIOUSLY SUPPORTED PROJECTS

### RESEARCH PROJECTS 2014

- Refinement of animal models of pain: Developing methods to alleviate pain in laboratory-rat pain research **COMPLETED**
- Pathological and immunological consequences of blood sampling in mice **COMPLETED**
- Standardizing gut microbiota in mice as a tool for reducing the number of animals in the individual experiments **COMPLETED**
- "Artificial blood vessels" – a model for investigating diabetic arteriosclerosis **COMPLETED**

### RESEARCH PROJECTS 2015

- Artificial skin in a Petri dish as an alternative to laboratory animals **COMPLETED**
- Developing an in vitro method to predict acute pulmonary toxicity from aerosol proofing products **COMPLETED**
- Can chickens be immunized with an aerosol combined with vaccination? Investigating a non-invasive method for producing antibodies

**Please note:** Further details about all 3R projects are available on the Danish 3R-Center's website [3rcenter.dk/forskning/forskningsprojekter/](http://3rcenter.dk/forskning/forskningsprojekter/)

## THE DANISH 3R CENTRE'S BOARD AND SECRETARIAT



PHOTO: KIM GRANLI

The seven members of the board: **Christine Nellemann** (chairwoman), **Peter Bollen**, **Axel Kornerup Hansen**, **Lisbeth E. Knudsen**, **Jan Lund Ottesen**, **Erwin L. Roggen** and **Adrian Smith**. **Peter Bie**, Animal Experiments Inspectorate, is affiliated with the board as an observer.

The board of the Danish 3R-Center is appointed by the Minister for Food and simultaneously makes up the National Committee for the Protection of Animals used for Scientific Purposes.

**Please note:** Further details about all board members are available on the Danish 3R-Center's website.



## THE FIRST FOUR YEARS FOR THE DANISH 3R CENTER

We of the board of the Danish 3R-Center are satisfied with the following achievements: we have supported a total of 13 research projects following a thorough assessment process; we have prepared a website, sponsored annual symposiums, granted 3R awards and drawn up teaching materials for lower-secondary and upper-secondary schools, and we have surveyed the perception of the 3Rs in Denmark.

As a result of these efforts, we feel that we have made great strides in supporting research in the area of the 3Rs and in broadly disseminating knowledge to the rest of society, which is notably the mission of the Danish 3R-Center.

In addition, as a national committee, we have articulated our expectations of the animal welfare bodies in Denmark, gathered them at annual meetings and taken part in drawing up guidelines for selected procedures involving laboratory animals in cooperation with the Animal Experimentation Council.

In our next term, we wish to continue the rewarding activities for which the Danish 3R-Center is already renowned, and we will continue our efforts to develop our international network, make contact with other foundations, funds and bodies that grant research funding and many other initiatives in keeping with the mission of the centre.

An additional possibility is to provide advice to users of laboratory animals on replacement and on an integrated testing strategy involving the use of cell models, computer systems and animals to make it possible to obtain more information from each procedure and presumably heighten the level of research conducted in Denmark.

It is our hope that we will receive lots of good input from all stakeholders and friends of the Danish 3R-Center, and we feel assured that the Danish 3R-Center will also be able to achieve excellent results in the period ahead.

Kind regards  
**The Board**

## THE DANISH 3R-CENTER'S SECRETARIAT

The secretariat of the Danish 3R-Center attends to the day-to-day tasks of the centre, from the ongoing tasks such as planning and holding of board meetings, symposium planning, news gathering and reporting, website operation, administration of research applications, information tasks relating to laboratory animals and the 3Rs and much more besides. The secretariat also launches the projects which, at board meetings, the board decides should be initiated.

## OVERVIEW OF DENMARK'S ORGANIZATION FORSØGSDYRSMÆSSIGE ORGANISERING

The following depicts the organization of the laboratory-animal area under the Ministry of Environment and Food.

### DANISH VETERINARY AND FOOD ADMINISTRATION

#### ANIMAL WELFARE & VETERINARY MEDICINE

- The Animal Welfare Section
- The Veterinary Medicine Section
- The Section for Research, Information, and Laboratory Animals

#### THE ANIMAL EXPERIMENTATION COUNCIL

The Animal Experimentation Council assesses every single application for carrying out animal experimentation in Denmark. Applications are discussed at Council meetings where the Council decides whether the application can be accepted or whether further explanation is required before a decision can be made. The Council members also take part in inspections of laboratory facilities and experiments.

#### THE ANIMAL EXPERIMENTS INSPECTORATE

The Animal Experiments Inspectorate processes applications for animal experimentation and conducts inspections of all animal experimentation facilities in Denmark. The Animal Experiments Inspectorate also provides advice on accommodation for and the use of laboratory animals, as well as on laboratory animal legislation in general.

#### THE NATIONAL COMMITTEE FOR THE PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes orders Member States to set up a national committee for the protection of animals used for scientific purposes.

This committee must advise the competent authorities (the Animal Experiments Inspectorate) and animal welfare bodies on matters concerning the acquisition, breeding, accommodation, care and use of animals for experimentation and ensure the sharing of best practices to promote the use of the 3R principles (Replacement, Reduction and Refinement) relating to animal experimentation. The committee chairperson and the other six members are identical to the board of the Danish 3R-Center.

Thus, the existence of the committee is required by law, whereas the Danish 3R-Center is a national initiative whose purpose is not only to provide advice but also initiate and disseminate information about 3R research and results.

#### THE DANISH 3R-CENTER

The Danish 3R-Center is a partnership between the Ministry of Environment and Food of Denmark, the Danish Animal Welfare Society, The Cooperative Body of Danish Animal Welfare Organizations (DOSO), LEO Pharma, Lundbeck and Novo Nordisk. The Danish 3R-Center works to promote the 3Rs in Denmark to bring focus to bear on alternatives to animal experimentation and create even better conditions for laboratory animals.

## MILJØSTYRELSEN

### PRELIMINARY ASSESSMENT OF REGULATORY RELEVANCE (PARERE)

The European Member States must designate a contact person (in Denmark a named individual in the Danish EPA) who must provide advice concerning the legislative relevance and suitability of alternative approaches proposed for validation. The PARERE network is affiliated with EURL-ECVAM, which is the EU's reference laboratory for alternative methods to animal experimentation.



PHOTO: CHRISTINA HAUSCHILDT

## TEACHING MATERIALS ON LABORATORY ANIMALS AND THE 3RS

### UPPER SECONDARY SCHOOLS

**[3rcenter.dk/forsoegsdyr/undervisningsmateriale-om-forsoegsdyr-gymnasiet/](https://3rcenter.dk/forsoegsdyr/undervisningsmateriale-om-forsoegsdyr-gymnasiet/)**

In a partnership with upper-secondary school teacher and freelance reporter Aiko Sho Nielsen, the Danish 3R-Center has prepared teaching materials on laboratory animals and the 3Rs aimed at upper-secondary education in the subjects of biology and biotechnology.

The teaching materials comprise an introductory quiz, a background article, a film, assignments, a concluding quiz and a teacher's book.

### LOWER SECONDARY SCHOOLS

**[3rcenter.dk/forsoegsdyr/undervisningsmateriale-om-forsoegsdyr-grundskolen/](https://3rcenter.dk/forsoegsdyr/undervisningsmateriale-om-forsoegsdyr-grundskolen/)**

The teaching materials for lower secondary classes 8 through 10 came into existence in cooperation with schoolteacher Amanda Jespersen and are based on the Danish 3R-Center's above-mentioned teaching materials for upper secondary schools.

The materials consist of an introductory quiz to be taken before starting the learning segment, a background article with tasks to be solved during the process, a resource space with links to be used for group work and a final quiz which shows the teacher whether the students' attitude to animal testing has changed and whether they have acquired knowledge of the subject.

The Danish 3R-Center publishes statistical information about laboratory animals which can supplement the above-mentioned materials, and the information can also be used in students' assignments, papers, etc. **[3rcenter.dk/forsoegsdyr/](https://3rcenter.dk/forsoegsdyr/)**

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## THE DANISH 3R CENTRE IN BRIEF

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### THE DANISH ANIMAL WELFARE SOCIETY

“The Danish Animal Welfare Society believes that the use of laboratory animals should be minimized. Therefore the Society actively supports the work of the Danish 3R-Center to replace, reduce and refine the use of laboratory animals. In particular, we have great confidence that efforts to promote the development and knowledge of alternatives to laboratory animals will contribute to realizing DAWS’ ambition to phase out the use of laboratory animals.”

### THE COOPERATIVE BODY OF DANISH ANIMAL WELFARE ORGANIZATIONS (DOSO)

“The overarching aim of DOSO is to abolish animal experimentation. Towards this end, DOSO is actively engaged in promoting the development, validation and implementation of alternatives to animal testing based on the 3R principles. Making an active effort and supporting the Danish 3R-Center improve our possibility of achieving this goal.”

### LEO PHARMA

“Many of our tests and trial models for developing medicines for treating skin ailments have been replaced in part by laboratory testing. However, we still need to use laboratory animals in the development of medicine to reliably assess the efficacy of medicines and comply with regulatory requirements. LEO Pharma’s animal welfare policy is focused on the 3Rs and we have initiated measures to reduce, replace and refine the use of laboratory animals in so far as possible. It is consequently a natural element of this policy to support the national 3R-Center to increase resources within the 3Rs.”

### LUNDBECK

“To facilitate the development of safe and effective medicinal products, we have to use laboratory animals at Lundbeck. It is our aim to continuously optimize the conditions for these animals and we use alternative methods whenever possible. For this reason, it was a natural choice for us to support the Danish 3R-Center so that we can stay abreast of 3R developments. With this collaboration, we support the continued development of all 3Rs while staying up-to-date on new ideas and initiatives that can benefit our laboratory animals.”

### NOVO NORDISK

“It is not yet possible to develop medicine that is effective and safe for patients without using laboratory animals. Novo Nordisk and the Danish 3R-Center share a desire to promote the development of alternatives to animal experimentation; reduce the use of laboratory animals; improve conditions for laboratory animals and communicate knowledge of alternatives to animal experimentation. Novo Nordisk makes an active effort to achieve these goals, which is why the company actively supports the Danish 3R-Center.”