

THE DANISH 3R CENTER IN BRIEF

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 the 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.

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 experiments; reduce the use of laboratory animals; improve conditions for laboratory animals and communicate knowledge of alternatives to animal testing. Novo Nordisk makes an active effort to achieve these goals, which is why the company actively supports the Danish 3R-Center.”

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ANNUAL REPORT THE DANISH 3R-CENTER

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ANNUAL REPORT
THE DANISH 3R-CENTER

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CHAIRWOMAN'S FOREWORD

DEAR ALL

2019 was an eventful year for the Danish 3R-Center. Our annual international symposium attracted a record-setting number of participants, including quite a number of international guests seeking to share their knowledge with us and learn more about our current research. It is pleasing to know that the Danish 3R-Center's work has an impact outside Denmark as well.

The 2019 3R Prize was awarded to Thomas Bertelsen of Novo Nordisk for his long-standing efforts targeting laboratory animals and those whose work involves laboratory animals. Thomas' work on and commitment to *Culture of Care* impressed the board in particular. His *Culture of Care* efforts have also put Denmark on the 3R world map. Therefore, I would like to take this opportunity to congratulate Thomas once again for winning the prize.

Another recurring event is the annual allocation of research funding to the 3R area where we always look forward to reading the many applications as they reflect a deep commitment in the field. The board is keenly interested in continuing to receive such large numbers of qualified, relevant research applications.

The Danish 3R-Center was established for the sake of the laboratory animals, which is why it makes good sense to heighten awareness of the 3Rs – *replacement, reduction and refinement* – in laboratory animal settings. Scientific progress has opened up options for contemporary researchers that were not available in the past, enabling them to focus much more on non-animal methods as the primary option.

Accordingly, we of the Danish 3R-Center have articulated a statement paper that describes this situation. Thus, in our work going forward, the Danish 3R-Center will endeavour to raise awareness of not only the 3Rs in laboratory animal settings but also of non-animal methods so researchers who use animal models will think along other lines and use different methods.

This year, we once again invited an external company to talk about its efforts focusing on the 3Rs. In this annual report you can read about the 3R activities at LEO Pharma. We are very pleased that LEO Pharma accepted this invitation.

I would take this opportunity to thank my fellow board members and the secretariat for their great dedication in 2019. It has been a pleasure working with you.

Last, but not least, I wish to express my gratitude to the Danish Animal Welfare Society, DOSO, LEO Pharma, Lundbeck, Novo Nordisk and the Ministry of Environment and Food for supporting the Danish 3R-Center in 2019. All these parties have stated that they intend to continue their financial support of the Danish 3R-Center, as they are satisfied with our work. Their support enables us to continue creating a leading environment for communicating information about how to use the 3Rs for the benefit of both research and laboratory animals.

Best regards

Christine Nellemann

Chairwoman of the board of the Danish 3R-Center

THE DANISH 3R-CENTER THINKS THAT A BROADER UNDERSTANDING OF THE CONCEPT OF REPLACEMENT OF EXPERIMENTS ON ANIMALS IS NEEDED

Sixty years have passed since William Russell and Rex Burch introduced the 3Rs in *The Principles of Humane Experimental Technique* (1959). The principles have been particularly successful, considering that they are currently integrated into animal research legislation in many countries. Quite a number of scientific reports conclude that the volume of information currently obtained per laboratory animal used is far greater than was the case in 1959, which is one of the fundamental features of *Reduction*. The housing of laboratory animals has also vastly improved and procedures have become more humane, resulting in *Refinement*. In addition, a number of routine tests for safety assessments of chemicals and drugs have been superceded by non-animal methods, leading to *Replacement*.

Approval procedures for new chemicals and medicines have also begun to utilise non-animal methods. This is exemplified by the adoption by the OECD (which harmonises testing guidelines) of the *Adverse Outcome Pathways* concept, specifying guidelines for mechanistic studies without laboratory animals. The Danish 3R-Center expects to see great benefits from sharing of experimental findings to maximize the level of knowledge. Yet research itself has changed, too, and research results are in far greater demand today than previously. Basic research at universities and the initial develop-

ment of new candidate drugs are areas that are particularly increasing in scope. These types of research are based far less on routine trials that repeatedly use the same laboratory set-up. Accordingly, there is still a great demand for targeted projects to implement the 3Rs, and for research into non-animal methods to make it possible for the more formative experiments in basic research to be replaced with non-animal methods as well.

Basic research and development is usually founded upon on a scientific question that seeks to be answered, after which an experiment that can answer the question is planned from scratch. Since no one else has probably answered the question previously, there is usually no previous experiment to which a new experiment can be considered to be a direct alternative. Therefore, the use of non-animal methods will largely be dependent upon the researcher, and not least the researcher's organization, who have multiple options when attempting to answer a research question. This means that a researcher will not only tend to think along the lines of animal research. The entire palette of research methods, including cell cultures, computer simulation and interdisciplinary analyses of research data, should therefore be considered whenever research questions need to be answered in vasic research.

Replacement vs non-animal methods

There seems to be some uncertainty about the concept of *replacement* in laboratory animal milieus. Therefore, it may be relevant to attempt to clarify the issues inherent in *replacement and non-animal methods*, respectively.

Replacement:

Russell and Burch defined replacement as follows: Replacement means the substitution for conscious, living higher animals of insentient material.

The Danish 3R-Center's definition: *Replacement of conscious higher animals with an ability to perceive external stimuli with insentient material, i.e. without an ability to perceive external stimuli. Replacement entails substituting experiments on live animals, covered by the Animal Research Act, with experiments that do not use whole, live vertebrates.*

Thus, *replacement* involves the actual substitution of an animal research model, which is why *replacement* is only an option for someone who already conducts experiments on animals. It is only by using an animal-based model that the user of laboratory animals becomes aware of possible alternative methods (*replacement*) to the animal model or that he/she attempts to develop an alternative method (*replacement*) to his/her animal-research model.

If the researcher successfully develops such a model, and it is otherwise validated, the method will now be deemed a non-animal method, i.e. it will no longer be considered *replacement*.

Non-animal method:

When a researcher is about to engage in a research task relating to animals, people or the environment, he/she should select the best model available, whether this is a non-animal method or an animal model (If an alternative *replacement* to the animal model exists, this must obviously be chosen).

One or more non-animal methods can supplement one or more animal models, thereby reducing the use of laboratory animals. Each individual method/model provides the answer to smaller parts of the overall research task.

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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.

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.

PRIORITY IS GIVEN TO PROJECTS'

- Quality
- Implementability
- Relevance

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.

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

RESEARCH PROJECTS SUPPORTED IN 2019

In 2019, the Danish 3R-Center received 21 applications for support, of which the following three received a total of DKK 1.5 million.

IMPACT OF SHELTER ENRICHMENT OF METABOLIC CAGES **IN STUDIES OF PROTEIN METABOLISM**

Helle Nygaard Lærke (Aarhus University)

Metabolic cage housing of laboratory animals is necessary within a wide range of nutritional, metabolic, pharmacokinetic and pharmacodynamic studies in both basic and applied nutritional and biomedical research. It allows accurate measures of food and water intake and urinary and faecal output, but restrained living space, use of grid flooring, absence of bedding substrate and enrichment, and social isolation impose negative behavioral and physiological responses. Earlier studies confirm that rats prefer a cage with a shelter, but such enrichment is challenging due to the risk of interference with the quantitative collection of faeces and urine. In nitrogen metabolism studies, there is an increased risk of N-loss via ammonia emission if urea in the collected urine is not trapped and acidified.

In this project, we will investigate if metabolism cages enriched with shelters can improve the animal welfare and be employed without interference with the quality of the quantitative data. Such data are important in order to implement improvement in nutritional and biomedical studies involving housing of experimental animals in metabolic cages.

GENETIC MODIFICATION OF MICE WITHOUT THE NEED FOR EXTENSIVE BREEDING

Per Svenningsen (University of Southern Denmark)

Cardiovascular disease affects a large proportion of the population and may arise because of changes in genes expressed in kidney and blood vessels. Genetically modified mice play a crucial role in understanding the underlying disease mechanisms and over the past 10 years, the use of genetically modified mice has increased by 40%. However, new figures have shown that 75% of the genetically modified mice produced by conventional methods are never directly involved in research, but instead they are only used to create and maintain the genetically modified mouse strains through extensive breeding programs. Therefore, there is enormous potential to reduce the number of mice used in research if we develop alternative methods to create genetically modified mice with no need for extensive breeding programs.

Gene modification can be created with adeno-associated vectors (AAV), which are virus-like particles comprising genetic DNA material surrounded by a protective protein coat. AAVs provide a safe and effective way to make gene modification in mice, but the current AAV-variants primarily target liver cells and therefore they cannot modify gene expression in kidney and blood vessels.

The AAV coat protein determines the target cells, and in this project, we will use a newly developed system

to create new forms of AAV coat protein, and develop AAV-variants that can effectively deliver DNA material to cells kidney and blood vessels. With the novel AAV-variants, we will accomplish a deeper understanding of the mechanisms that underlie the development of cardiovascular diseases while reducing the use of genetically modified mice.

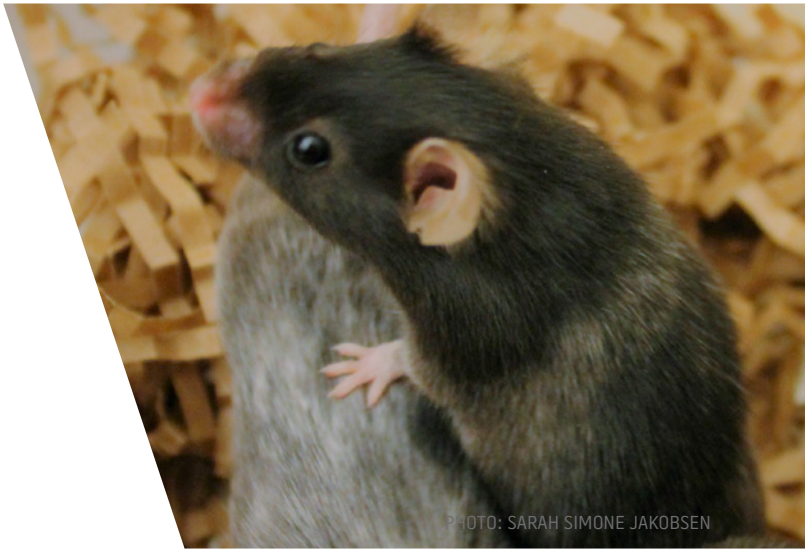


PHOTO: SARAH SIMONE JAKOBSEN

INVESTIGATION AND ANALYSES OF INTERNAL VALIDITY IN DANISH PRECLINICAL RESEARCH

Birgitte S. Kousholt (Aarhus University)

Animal experimental studies may provide important information that furthers biomedical knowledge and improves patient lives. However, animal experiments have poor reproducibility. There are most certainly several reasons why some experiments may be difficult to reproduce. One reason is low "internal validity". "The internal validity" refers to the extent to which the design and conduct of an experiment eliminate the risk of bias. If results are biased, clinical trials based on the results will then be based on skewed results. On top of that, the biased animal studies are a source of wasteful use of animals.

There are guidelines on how to plan and report animal experimental research to meet high standards of both research quality and code of conduct. These guidelines contribute to a reduction in the number of animals used in inferior studies. Unfortunately, there are only limited incentives to follow the guidelines. This may be because the internal validity of the animal experimental studies is generally not elucidated and communicated. In Denmark, there are no systematic data that illustrate the internal validity of animal experimental research.

This project uses a thorough, knowledge-based and systematic method, which will clarify if biased-limiting

incentives are reported. This is an indirect method used to extract to what extent the studies have incorporated measures which reduce the risk of bias. In addition, information on reporting of 3R initiatives is extracted.

This study is a responsible step in the identification of plausible deficits in implementing reduction in Danish in vivo research. Based on the results a targeted effort can be initiated to reduce biased animal experiments. This creates awareness, among all stakeholders, and the number of animal experiments which do not adequately use bias-limiting tools will decrease. The attention created will also strengthen the translational value of animal experiments. That is, the value needed to translate knowledge from animal experiments into scientific advancement and improved patient care.

OPTIMIZATION OF ORAL GLUCOSE TOLERANCE TESTING (OGTT) IN MICE

Dorte Bratbo Sørensen (University of Copenhagen)

The board of Denmark’s 3R Center, in connection to the application round, became aware of an exciting project in its very early stages focused on improving conditions for mice used for diabetes research. The board decided to establish contact with the researcher to hear more about the project and eventually grant financial support for the first part of the project.

The board found the project so relevant, it decided to support this part of this project with a smaller amount. It was agreed that an eventual support of the other part of the project could only be considered if there are positive results in the first part. Dorte Bratbo Sørensen concluded the first part of the project thusly:

In diabetes research, the oral glucose tolerance test (OGTT) is often performed on mice. The OGTT includes i.a. dosing with a glucose (sugar) solution via stomach tube followed by a series of blood samples obtained via tail vein blood sampling. It has been shown that tunnel handling of mice (i.e. the mice are lifted and moved while sitting in a small tunnel) reduces the overall level of anxiety among the mice and their fear towards humans. The purpose of this project was to design and test two devices that could make the OGTT gentler to the mice.

The first device was designed to allow blood sampling from the tail vein of the mouse, while the mouse remained in the handling tunnel throughout the process, including during the blood sampling itself. The device worked as intended and it can be used in combination with most standard handling tunnels.

The second device was developed with the aim of training the mice to be separated in the home cage and to voluntarily eat the amount of glucose solution normally administered during OGTT with a stomach tube. The mice were required to eat the glucose solution within a minute. Three different devices were tested in the process. The first device consisted of two tunnels, and the two subsequent devices consisted of a box of 1 and 4 chambers respectively. The 4-chamber device was selected as the design that was finally evaluated. In this context, it was also examined how the glucose solution could be made attractive to the mice to facilitate voluntary ingestion. Three formulations were tested: Glucose was offered to the mice either as a pure solution or mixed with either unsalted peanut butter or Nutella®. The mice showed the strongest preference for glucose solution when mixed in unsalted peanut butter. Pure glucose solution was preferred over Nutella® mixed with glucose solution.

However, the behaviour of the mice when separated was not compatible with eating behaviour, so it was not possible to achieve the expected intake of the peanut butter/glucose mixture within the time limit.

Never-the-less, several factors can be improved – including the time allocated for habituation and training of the mice; both in relation to the device itself and for the handling in connection to the dosing - and therefore it is likely that the device technique can be used after modifications for gentle and voluntary dosing of glucose in mice.

DECLARATION

JOINT EUROPEAN FUNDING PRINCIPLES FOR RESEARCH INVOLVING ANIMALS

Joint European Funding Principles for Research Involving Animals

Over the past two years, the Danish 3R-Center has initiated contact with a number of foundations in Denmark that issue grants to scientific and medical research to urge these foundations to accede to the international Joint European funding principles for research involving animals.

By acceding to the principles, the funders manifest responsibility relating to the use of research animals and help support transparency, sound research practice, better animal welfare in the research and better research results. You can see which organizations have acceded to the principles below.

Joint European Funding Principles for Research Involving Animals

The three key principles in brief:

- The funders recognize their role in promoting high-quality science and that good animal welfare and the 3Rs are integral to this.
- The funders aim to ensure that they only fund research using animals that implements the 3Rs.
- The European funders share good practice in terms of the funded research.
- The funders pool resources to improve animal welfare and scientific outputs in the funded research.
- The funders strive to ensure that the research funded is open and accessible wherever possible.

The following funders have acceded to the principles:

The Danish 3R-Center
Independent Research Fund Denmark
The Danish National Research Foundation
Gigtforeningen (Danish Society Against Rheumatism)
The Danish Heart Society
The Danish Cancer Society
The Lundbeck Foundation

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COMMUNICATION

The Danish 3R-Center prioritized its communication duties again in 2019. In addition to the centre's continued focus on maintaining a good website, the board decided to continue its emphasis of preceding years on *Science Calling*, as the Danish 3R-Center prioritizes communicating with schoolchildren for several reasons: First of all, it is important that the general discussion on laboratory animals is conducted on an informed basis, so that taking a position on whether one is for or against laboratory animals is rooted in reality. Secondly, we will find the researchers of tomorrow among schoolchildren, which is why the Danish 3R-Center considers it beneficial to give them knowledge of laboratory animals, non-animal methods, and the 3Rs early on.

The Danish 3R-Center has also taken part in the Danish Learning Festival and Naturmødet (National Nature Festival) in Hirtshals to promote the centre's teaching materials and raise awareness about the centre's work in general.

WEBSITE

An important tool in the Danish 3R-Center's efforts to raise awareness of the 3Rs is the centre's website (3rcenter.dk). The website communicates knowledge in Danish and English to animal-research and alternative-method environments, as well as to interested individuals. An overview of some of the contents relevant to researchers, animal technicians, animal keepers, schoolchildren, politicians, stakeholders and the general public is presented below.

The Danish 3R-Center's website

Researchers:

News from the 3R world

Information about relevant events (symposia, mini-seminars, animal welfare bodies' annual meetings, etc.)

Newsletters

Research funds

Research projects

The 3R Prize

Ressources for improving your research (e.g. PREPARE)

Articles about laboratory animals and experiments on animals

Presentations from 3R symposia organized by the Danish 3R-Center

Guidelines for implementing EU Directive 2010/63/EU

Links to teaching resources (for future users of laboratory animals)

Links to tissue-sharing services Annual reports

Animal technicians (animal keepers):

News from the 3R world

Information about relevant events (symposia, mini-seminars, animal welfare bodies' annual meetings, etc.)

Newsletters

Guidelines for implementing EU Directive 2010/63/EU

Links to resources specifically aimed for animal technicians

Annual Reports

Individuals/schoolchildren:

Teaching materials on laboratory animals and the 3Rs

Factual and basic information about laboratory animals:

- What are laboratory animals used for?
- How many laboratory animals are used?
- Which species are used in research?

Politicians and stakeholders:

Goals and goal-achievement forms for the Danish 3R-Center

Minutes of board meetings

Annual reports

SCIENCE PUB

One goal of the Danish 3R-Center in 2019 was to spread knowledge of laboratory animals and the 3Rs to the general public. This is one purpose of the 2019 event *Science Pub – an informal chat about science* (the Danish 3R-Center has previously held a similar event named *Pint of Science*).

Despite the new title, the concept was the same: an informal presentation in a cosy pub setting. Again this year, Axel Kornerup Hansen, board member, Danish 3R-Center, shared his knowledge of laboratory animals at a lecture entitled *Could the life of a laboratory animal be just a little more fun?* His topics included discussing the necessity of using laboratory animals, what can be done to make animal research gentler and how laboratory animals may become completely unnecessary someday.

Science Pub was held twice during 2019, at *Mikkellers Cafe Hyggestund* in Copenhagen and *Beer and Bottle Shop* in Odense, where participants listened to Axel's presentation over a cold beer. Both events were well-attended, but mostly by people who in one way or another relate to laboratory animals in either their studies or work.

A few individuals with no professional interest in laboratory animals chose to listen to the presentation. Apparently they thought it was interesting as they stayed and heard it all. Afterwards, people asked Axel questions which prompted an interesting debate.

The feedback after the event was quite positive and students in particular thought it was interesting to learn more about laboratory animals in an informal setting.

SCIENCE CALLING

WEEK 39

Each year, some of the Danish 3R-Center's board members give lectures as part of the *Science Calling* event, where Peter Bollen in particular has made a big effort as he visits a great many schools every year to talk about laboratory animals and the 3Rs.

Peter gives students a wide range of factual information about laboratory animals – such as the number of animals used in Denmark, the purpose of experiments on animals, which species are used, etc. – and introduces them to the 3R concept. In 2019, Peter gave his lecture to more than 600 schoolchildren at schools in central and western Denmark.

The Danish 3R-Center is considering whether to widen this effort in 2020 so that even more schoolchildren can gain insight into the world of laboratory animals and the 3Rs. The event also provides a good opportunity to promote the Danish 3R-Center's teaching materials about laboratory animals.

Science Calling

Science Calling is a lecture scheme under which scientists, students and others involved with science, technology and health visit schools to talk about a science topic based on their own field of interest.



LEARNING FAIRS

The Danish 3R-Center – in collaboration with the Animal Welfare Knowledge Center (ViD), which, like the Danish 3R-Center, belongs under the Danish Veterinary and Food Administration and has also developed teaching materials – took part in several events in 2019 to promote the centre’s teaching materials about laboratory animals and the 3Rs.

It is no exaggeration to say that the ViD’s teaching materials about animal welfare for grades 4–6 and 7–10 respectively went like hotcakes at the above-mentioned events. Unlike ViD, the Danish 3R-Center’s teaching materials for primary and lower secondary schools were available in digital form only, which made it more difficult to promote them. It was easier for ViD to draw attention to their materials because they could pass out printed copies which teachers could take with them.

The lessons learnt from these events has prompted the Danish 3R-Center to provide printed copies of its materials for lower secondary years 8–10, which will hopefully prompt even more schools starting to use the materials.

Promotion of teaching materials

- Danish Learning Festival (Bella Centre, March 2019)
- The Big Bang Fair (Odense Congress Centre, March 2019)
- Skolemessen (Aarhus Congress Centre, April 2019)
- Annual Meeting of Head Teachers (Bella Centre, November 2019)



NATURE FESTIVAL IN HIRTSHALS

23–25 MAY

The Nature Festival in Hirtshals is a different kettle of fish than the above-mentioned learning fairs. The reason the Danish 3R-Center’s takes part in the Nature Festival is primarily to raise general public awareness about the area of animal research and draw attention to the centre’s efforts to work for the benefit of laboratory animals – and not solely to promote its teaching materials.

The Danish 3R-Center took part in the Nature Festival for the second time in 2019. Festival attendees could visit the stand of the Danish Veterinary and Food Administration to talk about laboratory animals with secretariat staff from the 3R-Center which many people took the opportunity to do.

Schoolchildren and students were the main visitors on one of the Nature Festival days, providing a good opportunity to promote the centre’s teaching materials on laboratory animals.

Nature Festival in Hirtshals

The Nature Festival is an annual get-together about and in nature. Over the course of three days, Hirtshals is transformed into a centre for debates and experiences about Denmark’s nature

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3R ACTIVITIES AT NATIONAL RESEARCH INSTITUTIONS

In recent years, we have asked researchers from a number of external research institutions to describe their research and involvement with the 3Rs. In recent years, Aarhus University, Novo Nordisk and the Technical University of Denmark have contributed to this, just as three departments at the University of Copenhagen contributed articles to the last annual report.

This year, we asked LEO Pharma to provide input for our annual report.

3R EFFORTS AT LEO PHARMA

On Behalf of Research, **LEO Pharma: Kirstine Roepstorff and Janne Koch**

Previous external contributions

2015 Annual Report:

3R activities at Aarhus University

2016 Annual Report:

3R activities at Novo Nordisk

2017 Annual Report:

3R activities at the Technical University of Denmark

2018 Annual Report:

3R efforts at the Department of Animal Science (Aarhus University)
Work involving the 3Rs at the Large Animal Teaching Hospital (University of Copenhagen)
We use laboratory animals to strengthen the treatment of parasites in animals and people (University of Copenhagen)

Note: You can find all the annual reports here:
www.3rcenter.dk/om-3r-centeret/aarsrapport/

At LEO Pharma, we seek to integrate the 3Rs into the entire drug development process. From the early medicinal chemistry stages, we use *in silico* (computer based) tools to assess whether the substances designed are toxic. We do this via an experience-based computer database that recognizes toxic structures in substances. This enables us to reject substances that cause heart problems, for instance, before they are ever tested on animals.

Later in the drug development process, we test potential drug candidates/substances in cell-based systems. These systems are based on humane cells to which substances are admixed in different concentrations. Depending on the specific test, we can assess the efficacy of the substance in relation to the condition we want to treat or determine whether it has any deleterious side effects. In addition, we incubate the substance with liver-digestive enzymes from both rats and people to assess whether a given substance will be stable long enough in an organism to have the desired effect.

Once a substance has passed through the above-mentioned steps, in what we call the *screening cascade* for our candidate drugs, it is then fed to rats in a so-called pharmacokinetic study to assess the half-life/stability of substances in a whole organism.

Over the years, we have optimized our pharmacokinetic models for mice, rats and guinea pigs, so we can now restrict our efforts to taking *capillary micro samples* (CMS) from all three species during these studies. These 10 µl blood samples are taken from the tail vein of mice and rats and from the great saphenous or cephalic vein in guinea pigs. The tiny volume of blood makes it possible to take up to eight blood samples from an animal over a 24-hour period to get a complete pharmacokinetic profile from a single animal.

The CMS technique is possible only because our bioanalysis/DMPK department has developed an assay technique that enables them to analyse the concentration of the substance and its metabolites on the basis of 10 µl of blood. We hope to inspire other research institutions to implement the CMS technique as it reduces the number of animals used in an experiment and makes the blood sampling process gentler. In addition, we are in the process of implementing urine collection via LabSand in an experiment as an alternative to metabolic cages. If we succeed in validating this method as an alternative to urine collection in metabolic cages, this will greatly improve the animal welfare of these experiments, as keeping animals in metabolic cages is considered relatively stressful.

If the pharmacokinetic profile of a substance is acceptable, it proceeds to *in vivo efficacy studies* which investigate its ability to sufficiently reduce a provoked immune response in mice. Over the years, we have implemented the 3Rs wherever possible in our animal models. Examples worth mentioning are the shortening of several inflammation models from 22 to 14 days, when we proved that the so-called pharmacological window could be induced more quickly. We have introduced treats (mealworms, crushed maize or sunflower seeds) which we give to the animals after experimental procedures so they are distracted from any unpleasantness after the procedure.

In our daily involvement with the animals, we try to include the 3Rs in all activities (*Culture of Care*). We always clean the animals' cages when we have to handle them anyway as part of an experiment to avoid needlessly stressing them, and we always divide the animals into the groups they are kept in during the experiment upon returning home, to avoid subjecting them to additional stress by being divided into new groups before an experiment.

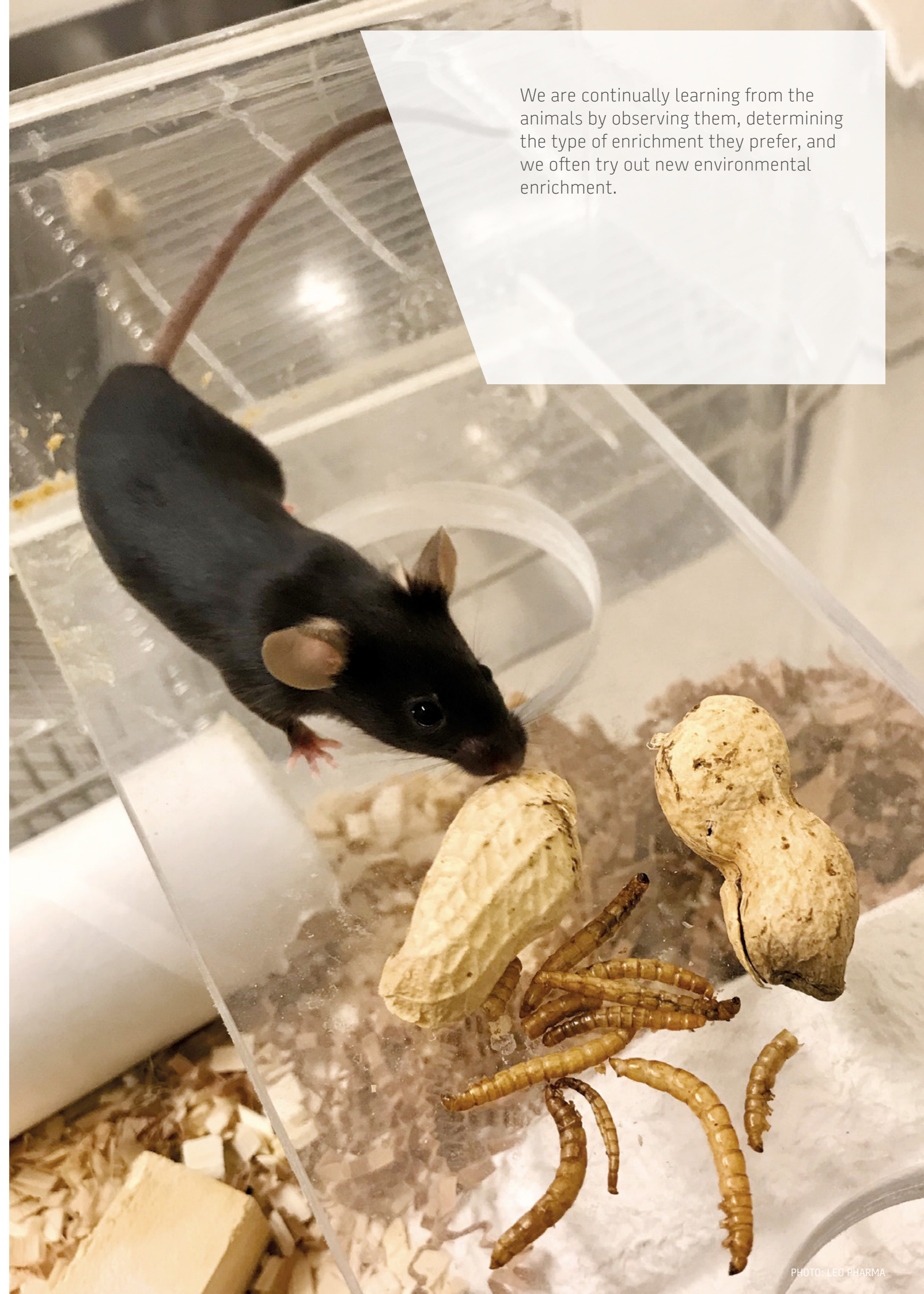
We also strive from the outset to house the lab animals in the room in which the experiment will be conducted to avoid unnecessary transport, and we always house our animals in relatively small groups to reduce the risk of fighting.

To improve our rats' conditions, we developed a new cage by modifying a traditional rabbit cage. This greatly enhanced their well-being. The rats exhibit calmer behaviour, and we observe them building nests, which we have not seen before in our male rats.

We are continually learning from the animals by observing them, determining the type of enrichment they prefer, and we often try out new environmental enrichment. For instance, we noted that giving our mice the option of climbing up in a tube suspended from the lid of the cage is a great source of enrichment for them.

At LEO Pharma, animal welfare is a matter of common interest. We take great pains to ensure that everyone involved in animal research actively takes responsibility for implementing the 3R principles. Besides the beneficial effect this has on animal welfare, these activities also promote employees' commitment and job satisfaction. Also, we believe that good animal welfare enhances the quality of our research data, because experiments conducted with unstressed, well-housed animals reduce the variability among the animals in the groups and improves their immune response.

We are continually learning from the animals by observing them, determining the type of enrichment they prefer, and we often try out new environmental enrichment.



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LABORATORY ANIMAL AND 3R EVENTS

To provide more details about 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 center itself.



WORLD DAY FOR LABORATORY ANIMALS, COPENHAGEN

24 APRIL

The World Day for Laboratory Animals is held every year on 24 April to highlight laboratory animals and the efforts to reduce the use of live animals as well as to improve conditions for the animals still being used.

Again this year, the World Day for Laboratory Animals was organized in collaboration between DOSO (the Cooperative Body of Danish Animal Welfare Organizations) and the Section for Experimental Animal Models (University of Copenhagen).

The theme for this year's World Day was the so-called *harm–benefit criterion*, which stipulates that the benefit of an experiment involving laboratory animals must be significant to justify the harm it incurs on the animals.

In his presentation, Leif Røge Lund, of the Animal Experiments Inspectorate, accounted for the work of the Animal Experimentation Council and Animal Experiments Inspectorate, whose discussions on notably *significant benefit* are worth highlighting in this annual report.

Leif Røge Lund focused on a number of projects deemed significantly beneficial by the Council to which it granted permission and explained the underlying reasoning of the decisions. Such discussions are obviously based on

the degree of stress to which the animals are subjected compared to the experiment's purpose/benefit.

One of the projects dealt with the use of fish and involved experiments in infection, pathology and immunology. The purpose of the project was to combat illness and identify treatment and disease-mitigation strategies in aquacultures. The results had the potential to improve animal welfare in the production process and reduce mortality and financial loss in the fish-farming business. The results could lower the environmental impact and the use of medicine and ancillary substances in the sector. Although it was necessary to subject the fish in the experiments to significant strain, the Council deemed the project's potential results significantly beneficial, and therefore gave the project the go-ahead.

As not all laboratory animals are used to benefit people, we want to mention an example presented by Leif Røge Lund, which had the potential to benefit animals, in this case dogs with cardiovascular disease and diabetes mellitus. The purpose of the project was to improve the treatment of the above-mentioned disorders, as well as further understand the underlying disease mechanisms in dogs. The Council deemed this project significantly beneficial, which also solely involved the blood-sampling of dogs, which were therefore “only” subjected to slightly stressful procedures.

For which purposes may animals be used for experiments?

1. disease prevention, poor health or other abnormality and the effects of this in people, animals and/or plants, including the production of drugs, substances and products, as well as the testing of their quality, efficacy and reliability;
2. disease diagnosis and treatment, poor health or other abnormality and the effects of this in people, animals and/or plants;
3. assessment, detection, adjustment or change of physiological conditions in people, animals and/or plants;
4. environmental protection;
5. improving animal welfare and improving production conditions for animals bred for agricultural purposes;
6. research, including basic research and research aimed at species preservation;
7. teaching and education at universities and institutes of higher learning or at any other educational process of similar level and for the training of individuals whose work will include experiments on animals; or
8. forensic investigations.

Training animals can give them a sense of autonomy and self-determination, as it can add predictability to their daily lives, etc.



PHOTO: THE DANISH 3R-CENTER

Another lecture held on the World Day for Laboratory Animals was given by Dorte Bratbo Sørensen, University of Copenhagen, entitled *Is the implementation of 3Rs equivalent to Animal Welfare?*

One of interesting reflections Dorte presented in her lecture was whether simply complying with the 3Rs is enough for the animals. Dorte asked the following questions: *“Do we need more than the 3Rs?”* and *“Should we focus more on the ‘good things in life’?”*, where the good things involve not only better cage enrichment that gives the animals a certain number of choices in their everyday activities, and thus helps improve animal welfare, but also the amenities which benefit animals when staff make an extra effort, such taking the time to learn how to tunnel-handle or train the animals. Such extra efforts have other positive add on effects – not just for the animals, but also for staff interacting with the animals. Training animals can give them a sense of autonomy and self-determination, as it can add predictability to their daily lives, etc.

Dorte’s focus on going beyond compliance with the 3Rs is well in keeping with the keener attention in *Culture of Care*, which notably involves striving to continually improve the existing efforts vis-à-vis laboratory animals.

The Animal Experimentation Council

The Animal Experimentation Council assesses every single application for carrying out experiments on animals 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 Council is made up of 11 experts from relevant technical fields. The Council members are appointed by the Minister for Food and Environment. The Council’s chairperson must be a judge.

In addition, one member is appointed on the basis of a recommendation issued by each of the following bodies: the Danish Council for Independent Research, Medical Sciences; the Danish Council for Independent Research, Technology and Production; the Danish Health Authority; the Confederation of Danish Industry; the major disease-combating patient’s associations; and the Danish Animal Ethics Council. The remaining four members are appointed on the basis of the recommendations of animal protection societies.

The members are appointed for four years at a time.

ANIMAL WELFARE BODIES' ANNUAL MEETING, COPENHAGEN

9 MAY

Each year, the National Committee for the Protection of Animals used for Scientific Purposes (UFA) organizes a meeting for members of Denmark's animal welfare bodies to discuss the function of these bodies. The meetings have been successfully expanded so that the programme for the animal welfare bodies takes place before noon, while the meeting is opened up to everyone with a professional interest in laboratory animals in the afternoon.

The by now familiar and well-attended *marketplace* is also held in the afternoon and is a forum where participants present and discuss the 3R initiatives they have brought with them and which each of them has successfully used in their respective laboratory animal facilities. This part of the meeting clearly illustrates that not only Danish researchers are intensifying their focus on the 3Rs. For instance, Denmark's animal keepers are enormously interested in continually improving the conditions of laboratory animals, which is abundantly expressed in the *refinement ideas* they present to one another at the *marketplace*.

The theme of this year's meeting was *Culture of Care*, which is why, in the same breath, we must mention Thomas Bertelsen's (Novo Nordisk) presentation at the annual meeting, due its great significance in raising awareness of precisely this important concept. Thomas Bertelsen was awarded the Danish 3R-Center's 3R Prize at this year's 3R symposium – for his significant efforts involving *Culture of Care* (described on p. 50). A few

of Thomas' bullet points on the role of animal welfare bodies in strengthening CoC are presented here:

- Together with the executive management, the animal welfare body must secure the implementation of suitable structures for promoting an expedient culture of care.
- Monitor the efficient delivery of results.
- Make all relevant staff familiar with the animal welfare body's role and urged them to contribute ideas and initiatives.
- Take a teamwork-minded approach, while maintaining authority at the same time.
- Encourage researchers to cooperate with animal keeping staff and appreciate their contributions;
- Communicate with all employees (performance/newsletters/website) and spread the message about the 3Rs, etc.

Moreover, Katrine Svendsen, Animal Experiments Inspectorate, used her presentation, *Culture of Care: Statutory Requirements*, to describe how the Inspectorate keenly focuses its inspections on active, well-established animal welfare bodies, as these play a major part in strengthening the culture of care at Denmark's animal research facilities.

Janne Koch, LEO Pharma, held a highly relevant presentation: *Animal welfare at foreign business partners: how do we make sure they comply with the EU Directive and internal standards?*



In the case of LEO Pharma, as much as 70% of its research activities are conducted by its partners, which is why the company's high internal animal-welfare standards can be compromised by business partners outside the EU.

Janne Koch exemplified this problem with specific examples. As part of an audit at a US business partner (contract research company), LEO Pharma ascertained that ferrets were being housed under conditions that did not meet LEO Pharma's standards. They were housed in cages with perforated plastic base plates and no enrichment, which is why LEO Pharma got involved in a constructive discussion to do something about this. This resulted in rubber mats being inserted into the cages, adopted to the base area of the individual cage, and bedding and hammocks being included in the cage fittings. At subsequent feedback from the partner, LEO Pharma was able to ascertain that both animals and keepers were enthusiastic about the modified cages, and that other customers had asked about the housing.

The above is a good example of how companies with high standards can inspire others.

An annually recurring point on the programme at the animal welfare bodies' annual meeting is the workshop, which is based on the annual meeting's topic, in this case *Culture of Care* (COC), and on a number of questions submitted by the steering committee which plans the annual meeting every year.

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.

2020 Annual Meeting of the Animal Welfare Bodies

The 2020 annual meeting was scheduled to take place on 11 June, but has been cancelled due to the COVID-19 pandemic. Sign up for the Danish 3R-Center's newsletter to stay up to date on a possible new date for the event: 3rcenter.dk/nyhedsbrevstilmelding/

It can be relevant to present some of the ideas which the questions prompted.

The questions were worded as follows:

1. What do you, as AWB members, think that you can do to raise awareness of and/or intensify focus on CoC at your workplaces?
2. What differentiates CoC from efforts to comply with the rule of law, in terms of both the 3Rs and animal welfare efforts in general? Are there any differences compared to minimum legal requirements, and, if so, what are they?
3. How can you, as AWB members, convince your colleagues, researchers and management that CoC is important and must be prioritized?
4. Does CoC require special resources, expertise or supportive structures?

In relation to *question 1*, it was mentioned that it is important to involve everyone who is in contact with laboratory animals in one way or another; and it is important to be alert to new employees entering the workplace with individuals attitudes about CoC, which need to be adapted to the new company's approach. Needless to say, a new employee can also have previous experience in this area, which can inspire his/her new company, as CoC is an eternal optimization process. Accordingly, it was also mentioned that continual attention must be given to developments in the field and inspiration must be actively sought from other enterprises. Similarly, one must be willing to dare to try out new ideas which must be approached with an open mind. At this point, it is worth mentioning that the most important purpose of Animal Welfare Bodies is perhaps to bring together people in the animal research area to let them inspire one another, including as part of the afternoon's marketplace event, which is notably an inspirational forum.

As regards *question 2*, it was mentioned that CoC, unlike the law, takes due account of the human factor and its significance, which is notably the most important factor for developing ideas and making an *extra effort*.

An interesting consideration for *question 3* dealt with the fact that better animal welfare also creates more job satisfaction for employees who interact with the animals, which is a good argument for establishing a culture of care.

Another important point during the discussion of *question 3* dealt with the fact that information and transparency, i.e. involving everyone, creates solidarity, which can heighten the interest of the individual employee in efforts to strengthen a culture of care. Conceivably, the fact that a researcher or veterinarian will make a bigger effort to get involved and listen to the animal keeper, who is crucial for animal welfare, will prompt the animal keeper to feel a deeper sense of recognition, which increases his/her dedication and job satisfaction.

Question 4 also led to some healthy reflections. It was emphasized that the support for and efforts to strengthen a company's culture of care require the individual employee to have a very positive attitude.

Conceivably, workplaces will not accommodate negative attitudes to the concept concurrent with the increasing integration of a *culture of care* into the basic values of animal-research companies.

Once again, it was mentioned that those employees who spend their daily work with animals – perhaps animal keepers and veterinarians in particular – have the best understanding of the animals' well-being which is why they play a key part in emphasising to management the necessity of prioritising both time and resources to strengthen a company's culture of care.

PREVIOUS WINNERS:

2017 **Josefine Hammer, Heidi Lehman and Janni Oxfeldt**
(DTU National Veterinary Institute)

2018 **Sara Mathez**
(LEO Pharma)

The winner of the 2019 3R competition for animal technicians

Upgrading of housing conditions and enrichment for rabbits, chickens and guinea pigs.

The year's winners of the 3R competition for animal technicians were Annett Christophersen, Bettina Ditlevsen, Lene Kirkegård, Natasja Friis, Thomas Olsen and Stine Louise Hansen from the Department of Experimental Medicine, University of Copenhagen, for their project 'Upgrading of housing conditions and enrichment for rabbits, chickens and guinea pigs'.

They won the competition based on the following project description:

Purpose of the idea:

"Even though all our buildings are outdated, we continue to house a variety of animal species, so we need to think along other lines to improve animal welfare and optimize their housing conditions.

All types of improvements must be custom-made, as our animal housing facilities have varying dimensions and unequal angles. There is virtually nothing we can purchase directly from a supplier, and everything has to be made by our technical/operating managers."

Description of the idea:

Guinea pigs: "Our guinea pigs are kept in open housing systems on open floors in cubicles we have made ourselves. We prefer a rectangular plate/covering at the end of the cubicle where the guinea pigs can run in and hide. It must be possible for the plate to be tilted up and down for cleaning and for catching the animals. Our shelter is not closed off at one end, so it does not give the animals a proper feeling of security. Installing the plate will enable more animals to find shelter and be further shielded from the light. This is particularly important as the animals in these experiments may not be given straw or other material to hide in, and the feeding of hay must be rationed."

Rabbits: "We want to use the same concept for our rabbits, as they will be able to use the plate to jump up on. For rabbits, we want to be able to establish digging compartments with sphagnum/chipped bark, so they can engage in natural digging behaviour."

Chickens: "We want to establish a 'sanctuary' for our chickens. A shelf with easy to clean plastic sheets where chickens at the lower end of the pecking order/bullied by other chickens, can hide and withdraw. And a roost with a sheet underneath onto which the chickens can defaecate."

FELASA, PRAGUE

10–13 JUNE

FELASA holds an international conference every three years. The 2019 conference was held in Prague and was the 14th in a row. The Danish 3R-Center was represented at the conference by the centre’s head of secretariat, a secretariat employee and four board members.

The four-day programme of events covered a wide range of topics, such as reproducibility in experiments on animals, humane end-points, pain treatment, tunnel handling, welfare assessment, culture of care, ethics committees, animal welfare bodies and the EU Directive on animals used for scientific purposes.

The FELASA conference featured an entire session on 3R centres. We are currently living in an era in which 3R centres area being established in many European countries at great speed. Many of the conferences on laboratory-animal science being held these days bring particular focus to bear on 3R centres, as was the case at the FELASA conference. Entire sessions devoted to the 3R centres gives new and old centres alike an opportunity to describe their structure, areas of focus and activities. Peter Bollen, Danish 3R-Center, took part at the 3R session in Prague with an oral presentation of the Danish centre. The 3R centres in Norway, Italy, Germany and Austria were also presented.

The efforts of the National Committee for the Protection of Animals Used for Scientific Purposes were also presented at the FELASA conference. This was done by means of a poster brought to the conference by Axel Kornerup Hansen, member of both the Committee and the 3R board.

All things considered, it was an interesting, informative conference where the Danish 3R-Center had ample opportunity to be updated on new knowledge, share information about the Danish centre and network with people from companies, universities and other 3R centres.

FELASA

The Federation of European Laboratory Animal Science Associations is an amalgamation of associations representing common interests in promoting all aspects of laboratory animal science within and outside Europe. Since 1978, FELASA has been instrumental in promoting responsible scientific behaviour relating to the use of animals in bio-scientific contexts with particular focus on ensuring a high level of animal welfare.

DANISH CANCER RESEARCH DAYS, ODENSE

29–30 AUGUST

In 2019, *Danish Multidisciplinary Cancer Groups* (DMCG.dk) and the *Danish Comprehensive Cancer Center* (DCCC) organized the Danish Cancer Research Days for the second consecutive year. The purpose of the event is to gather clinicians, researchers, patient associations and decision makers to share knowledge about cancer research and therapies.

The Danish 3R-Center took part in the conference with a stand, which also represented, among others, *the Danish Cancer Society Research Centre, the Danish Pathology Society, the Danish Society for Clinical Oncology* and various patient associations.

Two secretariat employees took part to raise awareness of the Danish 3R-Center, the 3Rs and the resources available from the Danish 3R-Center in the form of research support and materials for optimizing the use of laboratory animals.

Therefore, the Danish 3R-Center had brought along examples of some of the resources available on the 3R-Center’s website, such as annual reports, teaching materials about laboratory animals, posters with examples of materials for optimizing the use of laboratory animals (improve your research poster), etc.

An abundance of flyers with information on how to apply for 3R research funds were also handed out, and a number of conference participants admitted that the research funds could definitely be relevant for them to apply for.

Participants showed great interest in the Danish 3R-Center’s work, and the two secretariat employees spoke with many of the 500 participants at the conference. Many had never heard of the Danish 3R-Center nor of the many resources available from it. The reactions were very positive, and a number of people stated that they were glad the Danish 3R-Center was represented at the conference, which is why the Danish 3R-Center thinks it is relevant to take part at an upcoming event.

Several conference participants admitted that the research funds could definitely be relevant for them to apply for

EUSAAT, LINZ

10–13 OCTOBER

EUSAAT afforded several positive opportunities for the Danish 3R-Center, as the centre was not only featured on the programme with a presentation about the centre's support of 3R projects (by board member Lisbeth E. Knudsen, and with two posters about the work of both the Danish 3R-Center and the National Committee for the Protection of Animals Used for Scientific Purposes), but the centre also took part in a meeting of the *European 3Rs Centres' network*, which currently looks like it will develop into a rewarding collaborative network (read more about the network on p. 62).

A pointedly relevant presentation was held at EUSAAT by Susanna Louhimies, of the European Commission. The presentation dealt with increased openness/transparency about how animals are used in research (*Moving transparency to the next level – non-technical project summaries under Directive 2010/63/EU*).

Susanna's main message was that transparency is essential for engendering confidence in the general public that the care of laboratory animals and how they are used meets high ethical standards. The tools for doing this are the revised requirements for reporting on the use of laboratory animals, as well as the publication of non-technical summaries concerning projects that make use of laboratory animals (the non-technical summaries of the animal studies must make it possible for people without a scientific background to gain insight into a topic).

A status review of the directive, which was completed in 2017, showed a predominantly positive attitude to the transparency-friendly tools among EU Member States and users of laboratory animals, who felt that the tools served their purpose. Many representatives of animal protection organizations disagreed with this conclusion, however, as they were concerned about the accessibility of information, which they consider both difficult and slow. They also felt that the quality of the content was problematic and lacked a database at EU level in which to search for the non-technical summaries.

Susanna used her presentation to describe the work initiated by the EU Commission since then to accommodate the improvement proposals to improve the quality and accessibility, and she also indicated that a database with the non-technical summaries will become a reality.

In this context, it is worth mentioning that the Danish 3R-Center has always given high priority to communicating and publicizing information on laboratory animals, such as in teaching materials, lectures at schools, information on websites, etc. Similarly, for years Denmark has published all the permissions that have been granted for conducting of experiments on animals, to ensure transparency in the area of animal research.

At this year's EUSAAT, all of 77 posters were presented, many of which – despite the conference's generally keener focus on *reduction and refinement* – focused on *replacement* and non-animal methods. The posters on *Organs-on-Chips* (involving cells from skin, lungs, liver, etc., which can be used for toxicological testing of substances' efficacy), and on 3D cell cultures (which enable both basic research and toxicological testing in an interaction of multiple cell types, etc.) are worth mentioning here.

As previously mentioned, the Danish 3R-Center presented posters for both the Danish 3R-Center and the National Committee for the Protection of Animals Used for Scientific Purposes. The posters' creators had an opportunity to answer questions about the posters during the breaks between lecture sessions. Such sessions give the Danish 3R-Center a good opportunity to network with conference participants and promote its own symposium. Familiarity with the Danish 3R-Center's symposium has obviously increased in recent years, and a number of EUSAAT's participants praised the symposium and expressed a wish to take part in it.

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

THE DANISH 3R-CENTER'S SYMPOSIUM, COPENHAGEN

12–13 NOVEMBER

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

This year's moderator was Peter Bollen, member of the board of the Danish 3R-Center. He started out by welcoming the many participants to the annual symposium, which was the sixth of its kind, and expressed how pleased the board is with the record-setting attendance. After this, Peter introduced the first speaker: Lorna Ewart, Emulate.

The Danish 3R-Center's symposium is an annual international platform for researchers and national personalities with 3R expertise.



PHOTO: KIM GRANE

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

BEYOND ANIMAL TESTING: DEVELOPMENT OF ORGANS-ON-CHIPS TO EMULATE HUMAN BIOLOGY

Lorna Ewart, Emulate

Lorna Ewart of Emulate presented her company's work involving *Organs-on-Chips*, a technology that combines hardware and cultivated human cells to create the physiological and mechanical micro-environment of an entire organ. According to Lorna Ewart, it can function even better than animal models for safety assessments of chemicals.

Lorna told how the company had developed the micro-environmental conditions in multiple organs, such as lungs, liver, kidneys, intestines and brain. Below, you will find a film about the company's *Lung-on-a-Chip*, which brilliantly depicts its structure and functionality.

It is definitely possible that Organs-on-Chips can play a significant part in a non-animal future, even if this future isn't just around the corner.

Lung-on-a-Chip

This film was shown during Lorna Ewart's presentation and depicts how a lung chip is structured and functions.

Lung-Chip video link: <https://vimeo.com/267637620>

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

The Danish 3R-Center has successfully established a symposium of high professional standard.

THE DANISH 3R-CENTER – OUR TASKS IN 2019

Christine Nellemann, the Danish 3R-Center

Christine Nellemann, Chairwoman of the board of the Danish 3R-Center, began her presentation with the gratifying announcement that the 230 participants at this year's symposium are an attendance record. She also indicated her satisfaction with how the symposium has developed into an important knowledge-sharing and networking event over the years.

Christine was satisfied that the Danish 3R-Center has successfully established a symposium of high professional standard, evidenced by the top research experts who have held presentations over the years, one-third of whom have been speakers from outside Denmark.

Afterwards, Christine Nellemann introduced the symposium participants to the work conducted by the Danish 3R-Center during 2019. Christine started out by describing the recurring tasks, such as the annual report, website, granting of research funds and conference

participation, as well as tasks of a more one-off nature, such as working to establish cooperation among Europe's 3R centres, working to gather relevant 3R resources for use at Denmark's animal welfare bodies, organizing *Science Pubs* and participating in various events to promote the Danish 3R-Center's teaching materials on laboratory animals and alternative methods.

Christine concluded her presentation by urging the symposium participants to take part in next year's symposium which will be held on 12–13 November 2020.

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

PHOTO: KIM GRANLI

CODE OF CONDUCT FOR RESEARCH INTEGRITY

Marco Annoni, National Research Council, Italy

Marco Annoni gave a presentation on research integrity in which he explained how to interpret the concept and its significance.

Research integrity is about how the researcher not only aspires to scientific professionalism but also to 'morally' proper conduct. Marco gave a good example of this based on Watson and Crick, who, as everyone knows, won the Nobel prize for their discovery of the double helix structure of DNA. Marco's point was that Watson and Crick did not give credit to Rosalind Franklin whose research results had otherwise constituted the foundation for their discovery. This made them guilty – from a perspective of research integrity – of not aspiring to morally proper conduct by giving Rosalind Franklin the credit she deserved.

Marco Annoni gave another example of how research integrity had been ignored and which, unlike the Watson and Crick example, had consequences for public health.

In 1998, Dr Andrew Wakefield published a study in *The Lancet*, asserting a correlation between autism and the MMR (measles, mumps and rubella) vaccine. The research project undermined segments of public trust in the vaccine, after which many declined to be vaccinated. This ultimately led to a higher number of cases of both measles and mumps in the US and Europe. It later emerged that Wakefield and his colleagues had modified data about the children in the study and, to top it off, that Wakefield had been paid by a lawyer who was planning to sue the vaccine manufacturer.

When it comes to research integrity, it is important to remember that the researcher is not only responsible for his/her own reputation, but also for the reputation of the company with which he/she is affiliated and – as Marco put it – for the reputation of science and research in general.

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

PHOTO: KIM GRANLI

NEVER REPLICATE A SUCCESSFUL EXPERIMENT – FACING THE REPRODUCIBILITY CRISIS IN THE LIFE SCIENCES

Helene Richter, University of Münster

Helene Richter's presentation dealt with the so-called *reproducibility crisis*, which describes the fact that much research is either difficult or directly impossible to replicate, thereby raising questions about research quality and results. This means that, although important research results can also be provided by means of animal models, this problem can also adversely impact good research. For example, the use of animal models can lose its popular support if such problems overshadow the positive research results.

The causes of the reproducibility crisis are attributed to ordinary scientific inconsistency (poor scientific method), methodological and statistical pitfalls, etc. Recently, an additional potential source of poor reproducibility saw the light of day: endeavours to standardize experiments on animals, which has otherwise been considered a necessity.

If the variations in a specific experiment on animals are reduced, the standardization might restrict the results' relevance to identical experimental conditions. This

Much research is either difficult or directly impossible to replicate, which can also raise questions about research quality and results.

would ignore the individual characteristics that generate statistically significant results, which risk being irrelevant in biological contexts; also, the results cannot be replicated/reproduced under just slightly different experimental conditions.

The opposite of *standardization* is *systematic heterogenization*, which has been suggested as a possible solution for increasing the representativeness of a given study cohort, thereby increasing the results' external relevance and improving the prospects of replicating the experiment at the same time.

Another important point in Helene Richter's presentation in terms of strengthening research dealt with *preregistration* of laboratory-animal studies, which presumably have the potential to expose poor science. The concept simply means that before embarking on a study, the researcher publishes the purpose and methodology of the study, making it possible to prevent biases (in terms of publication, for instance) and data manipulation.

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

The percentage of applications focused on refinement has increased over the years, perhaps reflecting the Danish 3R-Center's focus on all the 3Rs as well as international 3R developments in recent years.

3R FUNDING OF RESEARCH ACTIVITIES 2014-2019 IN DENMARK BY THE DANISH 3R-CENTER

Lisbeth E. Knudsen, Danish 3R-Center

Lisbeth E. Knudsen, member of the board of the Danish 3R-Center, gave a presentation based on her study of the applications for research support submitted to the Danish 3R-Center since it opened.

on all the 3Rs as well as international 3R developments in recent years, which has shifted from primarily focusing on *replacement* to a keener focus on *reduction and refinement* as well.

Lisbeth described how 117 applications for research support had been submitted during the period 2014–2019, and that 19 of them had been granted support by the Danish 3R-Center. As part of the study, Lisbeth was particularly interested in finding out the fate of projects that had not received support. Therefore, Lisbeth used Pub Med to study whether these projects had ended up being published after all.

Lisbeth discovered that more than ten projects from the first two years (2014 and 2015) had ended up being published after all, which, according to Lisbeth, *might* indicate a heightened awareness of the 3Rs in general, not just by the 3R centres.

Lisbeth had also noted how the percentage of applications focusing on *refinement* has increased over the years, perhaps reflecting the Danish 3R-Center's focus

Note: Six of the year's presentations dealt with projects that had received support from the Danish 3R-Center. The presentations by Helle Nygaard Lærke, Per Svenningsen, Birgitte Kousholt, Rasmus Grønnemose, Axel Kornerup Hansen and Jes Dietrich are not mentioned here because their respective projects are discussed in Section 1 or the appendix.



PHOTO: KIM GRAN

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019

THE 3R PRIZE

Thomas Bertelsen, Novo Nordisk

The next item on the agenda was the awarding of the Danish 3R-Center's 3R Prize. The awarding of the annual prize is one of the Danish 3R-Center's highlights of the year, as it enables the centre to recognize an individual in the area of research who has made an outstanding effort in terms of the 3Rs.

Christine Nellemann, Chairwoman of the board of the Danish 3R-Center, began the session by explaining the board's justification for selecting Thomas Bertelsen, Novo Nordisk.

Christine explained that it was Thomas' genuine sustained enthusiasm for the welfare of laboratory animals over more than thirty years at Statens Serum Institut, LEO Pharma and Novo Nordisk, which made him an ideal choice for the 3R Prize. Thomas' contribution to Culture of Care had definitely been noticed by the board, and Christine expressed her gratitude for Thomas' always being very helpful and accepting, also vis-à-vis external colleagues.

After these words, Christine invited Thomas onto the stage to well-deserved applause from the audience before turning over the floor to Thomas, who talked about his work.

Thomas based his talk on *Culture of Care*, which for the past many years has been one of his areas of focus. (All the way back in 2006, Thomas organized the first joint meeting of animal welfare bodies in Denmark – now known as the *Private Network*.)

Thomas' work largely involves making an effort that goes beyond legislated practice, excellently exemplified by his efforts to promote a company's *Culture of Care*. Thomas described *Culture of Care* as the foundation on which various initiatives promoting animal welfare can be based, such as companies' 3R initiatives and the work of animal welfare bodies, the quality of which greatly depends on the viability of the culture of care.

Thomas' involvement in the field of laboratory animals for many years enables him to make fascinating observations of the developments that have taken place over the years. Thomas noted how non-scientific arguments, both ethical and moral, have gained wider acceptance in scientific circles. This trend is presumably crucial for establishing a culture of care, in which it is possible for employees to express remorse, such as during an experimental procedure, and (still) be met by acknowledging colleagues.

Winners of the Danish 3R-Center's 3R Prize

2014 Ellen Margrethe Vestergaard
The Danish Health Authority

2015 Hanne Gamst-Andersen
Novo Nordisk

2016 The QSAR team, represented by Eva Bay Wedebye and Nikolai Georgiev Nikolov
Technical University of Denmark

2017 Grete Østergaard
University of Copenhagen

2018 Birgitte Kousholt
Aarhus University

2019 Thomas Bertelsen
Novo Nordisk

The Private Network for animal welfare bodies

What is it?

- The Private Network for animal welfare bodies provides an opportunity to share knowledge.
- It does not duplicate meetings organized by the National Committee for the Protection of Animals Used for Scientific Purposes, but supplements them.
- The Private Network provides an opportunity for confidentiality. It is a forum in which it is possible to ask 'stupid' questions, without involving anyone else.

How?

- We organize knowledge-sharing meetings that are open to a wider group of members of animal welfare bodies.
- We can organize visits to one another for demonstrations of animal welfare initiatives.
- The members can contact one another internally with specific questions, etc.

- Basically, as many members of animal welfare bodies as possible should be able to take part in the events.

Who can join?

- The network is open to all members of Danish animal welfare bodies – regardless of education or position.

Sound interesting?

If you want to:

- actively work on tasks at your animal welfare body;
- be able to provide and receive knowledge about animal welfare;
- have an opportunity to give and receive feedback concerning ideas and problems – then you (your company/institution) should consider joining the network.
- Send an email to TSBT@novonordisk.com with the name of your company/institution, including contact person details (e-mail, tel. no., etc.).

The 3R Prize

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.

See more at: <https://3rcenter.dk/om-3r-centeret/3r-prisen/>

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019



PHOTO: KIM GRANL

Isn't it actually better science
that can improve the ethical
position of using laboratory
animals?

THE 3RS AND THE CREDIBILITY OF SCIENCE: ARE THEY LINKED?

Malcolm Macleod, University of Edinburgh

Malcolm Macleod gave an interesting and somewhat provocative presentation in which he questioned whether 3R compliance is necessarily beneficial from the perspective of laboratory animals and experimental results. For instance, Malcolm pointed out how it is often asserted that complying with the 3Rs leads to better science, to which he rhetorically asked whether an uncritical endeavour to do so could actually result in poorer science. For instance whether aspiring to minimise the use of animals (*reduction*) could result in using *too few* animals, which risks wasting the lives of the laboratory animals, if the experiment results are useless due to the low number of animals used. In this light, Malcolm rhetorically asked if it isn't actually better *science* that can improve the ethical position of using of laboratory animals?

Malcolm's point was that good science must form the underlying basis for the 3Rs in order for efforts to make sense, which is why Malcolm's presentation can be seen as encouraging everyone to start giving top priority to scientific method. In this context, he pointed out that most studies are not described with the abundance of detail necessary to enable studies to be replicated. Malcolm emphasized that it is not only the individual researcher who plays a crucial part in this, but scientific journals must also commit to initiating improvements.

When granting permission to use animals in research, it is essential that the world of research is also capable of presenting successful scientific breakthroughs to the public, thereby justifying the use of animals in research and strengthening the ethical position of the process.

If such a scientifically optimized use of laboratory animals embraces the 3Rs at the same time, this will benefit both science and the laboratory animals, all other factors being equal.

POSTER PRESENTATION

At the symposium, anyone who brings a poster also has an opportunity to present it, an option that will also exist for the 2020 symposium, by the way. We urge everyone who brings a poster to make use of this option.

Posters at the Symposium

- 3R: Dilution of PET tracers prior to preclinical imaging
- Better welfare through positive reinforcement training and VAB catheter
- Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS)
- Inhaled pharmaceuticals: Correlation between in vitro and in vivo lung effects
- Investigating the proliferative and serum-reducing effect (s) of various protein hydrolysates on 'Vero cells' as an ATMP bioproduction cells
- Mouse Kidney Parvovirus: A Newly Characterized Parvoviral Pathogen of Research Mice
- Pig training - a refinement, research and welfare initiative
- PREPARE before you ARRIVE: Guidelines for planning animal research and testing
- PREPARE guidelines for better Science (film)
- The Danish National Committee for the Protection of Animals Used for Scientific Purposes
- The Norecopa website: a collection of global 3R resources
- Tighter control of relative humidity improves murine breeding performance - a retrospective analysis
- Ultra-micro blood reduces the severity of the procedure and the number of animals used

Note:
You can find all posters on the Danish 3R-Center's website.

THE DANISH 3R-CENTER'S
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PHOTO: KIM GRANLI

THE 9 TO 5 RODENT – TIME FOR CHANGE?

Penny Hawkins, Royal Society for the Prevention of Cruelty to Animals (RSPCA)

Penny Hawkins questioned whether the use of animals in research – in this case mice and rats – is done on the staff's own terms when it comes to circadian rhythms. Obviously, there is a natural explanation for this, as employees use the animals during a typical working day, but this can be a problem for nocturnal animals (mice and rats) which are typically inactive in this time frame. As mice and rats make up the vast majority of laboratory animals, it is relevant to take a closer look at these factors.

Penny Hawkins explained that both the time of day and the laboratory's artificial lighting can significantly impact the behaviour and physiology of rodents, which can pose problems for animal welfare and scientific validity. For instance, it is conceivable that conducting behavioural tests during a period when the animals are naturally inactive could impair the results due to a cognitive deficiency, insufficient motivation to perform a

test or physiological reactions to the stress that arises from being disturbed during while resting.

If so, this must give cause not just for ethical concerns – as the results can be adversely affected and render the results useless – but animal-welfare concerns as well, if the animals experience the experiments as more stressful than if they had been conducted during the animals' naturally active period.

Penny explained how the effect of using animals in what is a period of activity for them remains to be adequately revealed, but it also makes sense in this respect to follow the precautionary principle and seek to minimize any factors that could impair research results and animal welfare (see the article *The 9 to 5 Rodent – Time for Change? Scientific and animal welfare implications of circadian and light effects on laboratory mice and rats* (Journal of Neuroscience Methods)).

THE DANISH 3R-CENTER'S
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SEX BIAS IN PRE-CLINICAL RESEARCH

Natasha Karp, Astra Zeneca

Natasha Karp questioned standardized preclinical trials – as did Helene Richter, by the way – in this case as they relate to sex bias by taking insufficient account of gender differences, thus rendering the results inadequately transferable to clinical experiments. Moreover, the gender aspect is yet another factor to consider in experiments that seek to replicate an experiment.

In this context, Natasha presented remarkable figures in her presentation. She explained that gender is not specified in the reporting of *in vivo* studies in the area of preclinical research; also, all of 80% of the studies in the same area solely involved the use of male rodents. Natasha supported this by presenting data showing that all of 80% of the drugs that were recalled from the US market in the period 1997–2000 posed a greater health risk to women than men, which seems to indicate that the unilateral use of male animals be a serious problem.

This unfortunate consequence of rejecting female animals in research has heightened the awareness of this so that gender will hopefully become a biological variable from now on in any study in which gender is relevant. It is worth noting here that increasing the use of females does not result in using more animals in general, as the individual researcher only has to replace half of the animals (males) that are otherwise used with females.

Gender is not specified in the reporting of *in vivo* studies within preclinical research

THE DANISH 3R-CENTER'S
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PHOTO: KIM GRANLI

Is the use of
animals in research
defensible?

HOW TO IMPROVE SCIENTIFIC VALIDITY AND ANIMAL WELFARE: GUIDELINES FOR ANIMAL RESEARCH

Adrian Smith, Danish 3R-Center and Norecopa

Adrian Smith, member of the board of the Danish 3R-Center, gave a presentation based on the long-standing discussion of whether experiments on animals are defensible, a discussion that to a greater extent today than previously also involves the researchers themselves who question the validity, reproducibility and translatability of the animal experiments.

In efforts to ensure both the transparency and high quality of the research, multiple guidelines have been drafted over the years for the reporting of experiments on animals but so far without any great effect, even if the most relevant publications endorse several of these.

In Adrian's opinion, it was doubtful whether the current debate about the *reproducibility crisis* is productive in terms of questioning customary practice and paving the way for a scientific approach that rests on a more informed, thorough and quality-controlled basis. Similarly the discussion can hopefully contribute to a greater understanding of how multiple factors can affect both the validity of results achieved through experiments on animals and the animals' well-being. According to Adrian, this understanding is essential for making harm-benefit assessments.

The debate concerning the *reproducibility crisis* seems largely to concern the mathematical aspects of experimental designs and statistical analyses, but unsatisfac-

tory results can also arise from detrimental conditions for the laboratory animals. Above all, proper conditions for laboratory animals – in terms of both housing and experimental procedures – require close collaboration between researchers and the animal technicians who provide the care and who conduct the experimental procedures.

Based on the above-mentioned factors, Adrian Smith et al. (Eddie Clutton, Elliot Lilley, Kristine Hansen and Trond Brattelid) have drawn up and recommended guidelines for how to plan experiments on animals. The guidelines are called PREPARE (Planning Research and Experimental Procedures on Animals): Recommendations for Excellence) and can be found on Norecopa's website (norecopa.no/prepare). The Danish 3R-Center highly recommends that these be followed when planning experiments on animals.

PREPARE guidelines

Norecopa has made a three-minute film about PREPARE, which is recommended by the Danish 3R-Center.

You can find the film here: vimeo.com/358069203

THE DANISH 3R-CENTER'S
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MECHANISM-BASED MATHEMATICAL MODELLING AS AN ALTERNATIVE TO ANIMAL EXPERIMENTS

Johanne Gudmand Høyer, Technical University of Denmark

Johanne Gudmand Høyer's presentation dealt with how mathematical models can also be applied to *replacement, reduction and refinement*, thereby improving animal welfare and reducing the use of laboratory animals. In this context, Johanne mentioned how it is possible to substitute some *in vivo* studies with *in vitro* experiments combined with a mathematical model, how biological hypotheses can be tested using models based on current biological knowledge, and how mathematical models can be used to select (prioritize) which experimental procedure is deemed most important, making it possible to avoid unnecessary experiments on animals.

Moreover, Johanne's presentation examined her work on mathematical models relating to the hypothalamic-pituitary-adrenal axis (HPA axis) which is responsible for the body's cortisol level. She explained how she mathematically models the relationship between the neuroendocrine system (HPA) and symptoms such as depression, which enables her to obtain information about this without having to use animals.

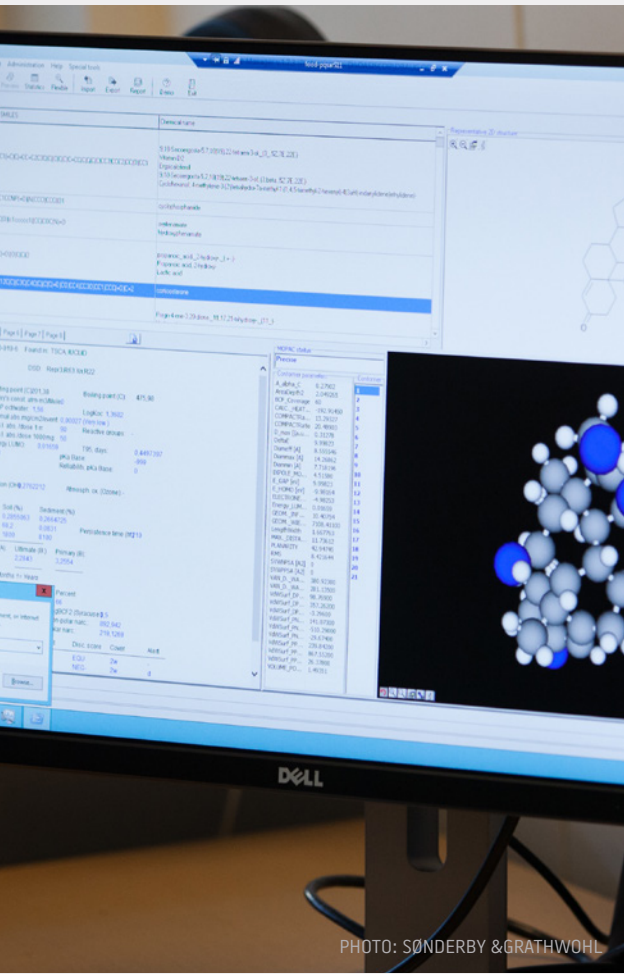


PHOTO: SØNDERBY & GRATHWOHL

THE DANISH 3R-CENTER'S
SYMPOSIUM 2019



PHOTO: KIM GRANL

HANDLING AND TRAINING FOR LESS STRESSED LABORATORY RODENTS

Camilla Bengtsson, RISE Research Institutes of Sweden

Many members of the audience could probably hardly believe their eyes when Camilla Bengtsson screened two films during her presentation showing how at RISE training was used to create a unique cooperative relationship between staff and laboratory animals (mice and rats). Their efforts are an almost perfect example of both *refinement* and a well-developed *culture of care*. From the very first day on which they receive the animals, RISE begins the task of engendering confidence in the facility staff and training the animals to take part in experiments which include procedures such as oral gavage and blood sampling.

The results are astonishing, which is why all users of laboratory animals are urged to see these two RISE films, in which it is obvious that both mice and rats take part in experiments with a high degree of voluntariness.

Obviously, this requires resources in terms of working hours to train the animals in this manner, but as animal welfare and valid research results go hand in hand, the time is definitely well spent, for ethical reasons as well.

Films: 'Mice at RISE' and 'Rats at RISE'

RISE has produced two short films on the handling and training of mice and rats, respectively. Find the films (*Mice at RISE* and *Rats at RISE*) on the 3R-Center's website:

3rcenter.dk/forskning/forbedr-din-forskning/

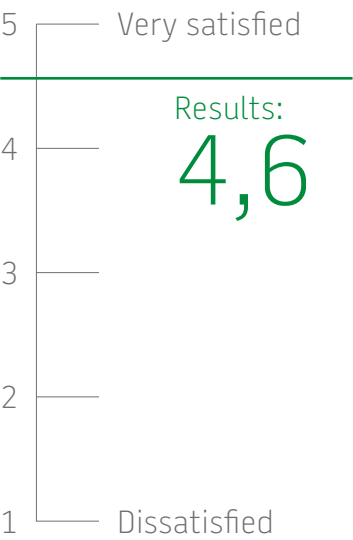
THE DANISH 3R-CENTER'S
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SATISFACTION SURVEY OF THE DANISH 3R-CENTER'S SYMPOSIUM

Eighty symposium participants were surveyed in the days after the symposium ended to determine their level of satisfaction with the event. Each participant was asked to rate his/her satisfaction (or lack thereof) on a scale of 1 (dissatisfied) to 5 (very satisfied). This resulted in an average rating of 4.6, making the 2019 symposium the highest rated symposium so far.

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 that it is worth pursuing in the future.

Average level of satisfaction



5

INTERNATIONAL COOPERATION

In 2019, the Danish 3R-Center continued to focus on international cooperation, as this is a logical part of our strategy for disseminating information about the 3Rs, considering the relatively modest size of our centre. In addition to the vast networking that arises 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, some of which will be described here.

Due to the vast differences of the members, the individual 3R organizations can perceive and prioritize the 3Rs (replacement, reduction and refinement) differently, which makes it important to agree on both the meaning and importance of the concepts.

THE EUROPEAN 3RS CENTERS NETWORK

One of the particularly interesting teamworking initiatives is the ongoing work to establish specific cooperation among the European 3R organizations. Winfried Neuhaus (President of EUSAAT) proposed establishing this network during EUSAT 2018, after which the number of networking members has grown, at the same time that the network's possible teamworking options are beginning to crystallize. In this context, the parties discuss their possibilities of cooperating on the following topics: Implementation of the 3Rs; Communication, Scientific Translatability; Teaching; Ethics.

In these efforts, both the Danish 3R-Center and Norecopa (Adrian Smith) have taken on some important tasks. Adrian Smith has posted an interactive map on Norecopa's website from where it is possible to access more than thirty 3R organizations, many of which have joined the network with a wish to strengthen cooperation (<https://norecopa.no/overview-of-european-3r-centres>). All 3R organizations are described on the website pages, thereby providing an overview of the individual organizations' core areas, which makes it easy to identify the potential for cooperation among the many 3R organizations.

The Danish 3R-Center has drafted a memorandum of understanding for the network. At present (early 2020), the draft memorandum has been sent to the network members for consultation. The memorandum of understanding must naturally provide the network, comprising 3R organizations with widely varying tasks and organizational strictures, with a foundation on which to base the cooperation.

Due to the vast differences of the members, the individual 3R organizations can perceive and prioritize the 3Rs (*replacement, reduction and refinement*) differently, which makes it important to agree on both the meaning and importance of the concepts. In this context, it is worth mentioning that the Danish 3R-Center has proposed focusing on *all* 3Rs, and also wants to intensify focus on non-animal methods (see the Danish 3R-Center's statement paper *"The Danish 3R-Center deems that there is a need for a wider understanding of the concept replacement of experiments on animals"* on p. 8 of this annual report).

CHARITÉ – UNIVERSITÄTSMEDIZIN BERLIN

The Charité university hospital, affiliated with the medical faculties at Humboldt-Universität zu Berlin and Freie Universität Berlin, recently established the 3R centre 'Charité 3R'. Together with the already existing BF3R, this gives Berlin a prominent place on the 3R world map.

As is the case with many other university hospitals, Charité conducts experiments on animals to develop and improve treatments of people. To maintain focus on the 3Rs during these experiments, Charité has established an internal 3R centre. The purpose is both to support 3R research and to train students and researchers in how to apply the 3R methods. Finally, the hospital has decided to disseminate knowledge of this by cooperating with other university and research institutes in Berlin and by communicating with everyone who is interested in the area. This includes practical training, symposia, conferences, websites and much more.

Besides a dedicated staff who are responsible for day to day operations and contacts to leading researchers in both Germany and abroad, Charité 3R has set up a *scientific advisory board* (SAB). Tom Bengtsen, Head of

Secretariat, Danish 3R-Center, has joined this board, which is why he took part in Charité's first international symposium in December 2019 and a subsequent board meeting.

In this context, Tom can draw on his vast experience of establishing a well-run 3R centre and can be a source of inspiration for various relevant tasks at the centre. Tom can also share the Danish 3R-Center's knowledge of how to best impart 3R knowledge to the centre's wide variety of stakeholders. As part of Tom's participation at the above-mentioned symposium, he gave a presentation in which he urged Charité 3R to prioritize its communication efforts and cooperate on this both in Germany and abroad.

The Danish 3R-Center is convinced that the close relations between the Danish 3R-Center and Charité 3R will benefit both organizations, both technically in terms of 3R and in the area of communication and dissemination. The Danish 3R-Center looks forward to developing this cooperation.

THE SWEDISH 3RS CENTER

During 2019, the Swedish 3Rs Center established an efficient, active organization which is apparent from its many scheduled events and the enhanced visibility in terms of news information on both social media, Twitter and in newsletters.

The secretariat held an informal meeting with Swedish representatives at the Danish 3R-Center during the annual 3R symposium in Copenhagen. At the meeting, both parties expressed a wish for closer cooperation.

The Danish 3R-Center has subsequently suggested that the centres should identify any identical tasks and use this as a possible basis for future cooperation. It was also proposed that the two centres' respective communication employees could hold one or more meetings a year to discuss how to cooperate on specific tasks.

That said, the two centres are already collaborating on disseminating news and promoting events by means of newsletters, Twitter and the centres' respective websites.

Even closer collaboration with the Swedish centre, possibly under the auspices of the above-mentioned European 3R Centres' network, is perhaps another option that the Danish 3R-Center will pursue.

Initially the centres should identify any identical tasks and possibly base future cooperation on this.

APPENDIX

On the following pages you can find a status update (as per the end of 2019) of the three projects supported by the Danish 3R-Center in 2018, followed by a list with all the projects that have been supported by the Danish 3R-Center since it was founded in 2013.

THE MOUSE PASSPORT (FINISHED PROJECT)

Axel Kornerup Hansen, University of Copenhagen

The mouse is the most widely used experimental animal – in Denmark and globally. In diabetes research, which is one of the largest research areas in Denmark, mice of the strain C57BL/6 are fed a high fat diet, which induces obesity and a diabetes-like condition. How many animals to use in each experiment is determined by how differently the mice react and how obese or diabetic they become. Recent research has shown that this variation is largely controlled by the gut microbiota of animals, which today, unlike previously, can be relatively easily fully characterized by sequencing on stools from the animals. Another influencing factor may be the animals’ genomics. Very similar inbred mice are mostly used in research, but recent methods have shown that the mice in their genetic material outside the coding genes may carry mutations, which at some breeders can be in high numbers. The non-coding areas of the genome control how high or low the different genes are expressed in the mouse.

In this project, each mouse has had their gut microbiota characterized, and in mice from a colony with a known

genetic variability, the mice have also been genetically characterized. For comparison, mice from a colony without genetic variability have also been used. The-reafter, an obesity study with a known treatment was carried out after high fat feeding. In the data evaluation, the mice have been grouped according to their genetics and intestinal flora, and it was calculated whether such a grouping, where the normally uncontrolled variation is now controlled, means that fewer mice can be used in a trial of this type.

The result was that the characterization of the mice, where each mouse was given a ‘passport’ on the basis of their individual gut microbiota and genetics, could significantly reduce group sizes.

TRANSPORT AND METABOLISM OF ANTIFUNGAL DRUGS IN THE HUMAN TERM PLACENTA

Bjarne Styrishave, University of Copenhagen

We have completed the experimental part of the project. We have actually had the opportunity to make some more perfusions on miconazole. This drug is a racemic mixture of R-miconazole and S-miconazole. We originally intended to only examine the racemic miconazole which is the therapeutic formulation for women, but we have achieved so many successful perfusions this autumn that we have also succeeded in making four perfusions each for both S-miconazole and R-miconazole, the two enantiomers of miconazole.

This is relevant as scientific studies indicate that the two miconazole enantiomers can exhibit different pharmacological and toxicological effects.

We have also made studies to identify whether the azoles accumulate in the placenta or only perforate through it. The studies show that fluconazole trans-ports easily through the placenta with an equilibrium arising at about 40% fluconazole on the maternal and the fetal sides, respectively. Of the remaining 20%, only 11% are accumulated in the actual placenta. This leads us to assume that only approximately 9% of the fluconazole is metabolized by the placenta.

The situation is different for miconazole. There is an equilibrium with less than 10% on each side, i.e. on the maternal and the foetal sides. About 30% accumu-late in the placenta, and the concentration is higher than for fluconazole. The remaining proportion of 60% cannot be recovered in the system and is consequently assumed to be metabolized by the placenta.

We are able to examine the prevalence of azole metabo-lites in the placenta, meaning that the above hypothesis of the placenta metabolizing the azoles can be tested.

We have also applied *Mass Spectrometry imaging (MSI)* to achieve a visual distribution of the azoles in the placenta. Fluconazole is distributed across the entire placenta, indicating swift and passive diffusion through the placenta. Miconazole appears to behave diffe-rently. Here, there is more miconazole on the maternal side than on the fetal side. We want to do more MSI imaging to get a more precise two-dimensional image of azoles in the placenta.

In the remaining five months of the project we will also analyse the prevalence of steroids in the placenta, as we assume that the accumulation of azoles in the placenta can affect the placenta’s endocrine functions. We will also perform qPCR on the most important CYP enzymes in the placenta which are involved in the regulation of steroid synthesis.

We expect to eventually end up with a fairly good overview of the transport of azoles through the human placenta. We also expect to get a good overview of drug distribution within the placenta, both quantitatively and qualitatively. We will also provide our sugges-tion as to the mechanisms with which the azoles can affect natural placenta endocrine functions and the potential implications of this on a pregnancy.

Also, we expect that our data will qualify for publica-tion in an peer-reviewed scientific journal.

NEW ADVANCED BLOOD INFECTION MODEL (FINISHED PROJECT)

Thomas Emil Andersen, University of Southern Denmark

Blood infections are a major problem compromising around 12.000 Danish cases each year, of who around 2.000 die. Blood infections occur, when microorganisms spread from e.g. wounds or the urinary tract to the bloodstream, where the microorganism can cause a serious infection such as septic shock or endocarditis. Today, research in blood infections and drug development uses simple cell culture-based laboratory models and animal experiments.

The problem with standard, cell culture-based models, however, is that they often are too simplified and only to a limited extend mimic the conditions in the human body. This means that results from these experiments often deviate from the following animal experiments and clinical trials.

In this project, we utilize our experience with biofilm and cell culture-based models under flow to develop a new advanced endothelial infection model that closely mimics the conditions of the human blood vessel. This enables detailed studies of blood infection including infections caused by the bacterium *Staphylococcus aureus*. This research is highly relevant, since this bacterium in particular is known for its virulence when spreading through the bloodstream.

In the project, we will focus on the optimization of the model to allow studies of biofilm formation under flow of human blood plasma both on blood catheter surfaces (the main entry of the bacterium in hospitalized patients) and on endothelial surfaces. For this, methods such as time-lapse microscopy are employed using fluorescent bacteria, blood components, and biofilm markers. The results will subsequently be compared with results previously obtained from animal models. It is expected that the model will improve the quality

of research results in relation to standard cell culture-based models, and also limit the use of animals in research and development at universities and in the industry.

Short status at the end of project

Laboratory models of blood infections are currently limited by being too simplified thus preventing them from replacing animal models. In this 3R-funded project, we have established a model that permits detailed studies of bloodstream infections, including infections of the heart valves. For this we have used blood vessel cells isolated from human umbilical cords, which we culture into a blood vessel epithelium layer in flow chambers. The blood vessel epithelium is then infected with bacteria, and by using microscopy and molecular biology methods, we can study how the bacterium establishes itself and penetrates the epithelium. This first step of metastatic spreading through the bloodstream and invasion of organs is a critical step during blood infections, and can with this method for the first time be studied directly and at close range during long-term experiments, without the use of animals.

The results are currently being compared with samples from a separate animal infection project, from which we will be able to see to what degree that our enhanced artificial blood vessel model can replace the use of animals. In this way, we will expect that our model can create new knowledge about bloodstream infections, which for example can be used to improve treatment, and furthermore to replace the use of animals for blood infection experiments. The results are currently being compiled in two articles about the gene expression of invading staphylococci and the pathogenesis of heart valve infection, respectively.



LIST OF
SUPPORTED PROJECTS
2014-2019

Descriptions of all the projects
can be found here:
en.3rcenter.dk/research/projects

PROJECTS	R	RESEARCHER
2014		
Establishment of an In vitro model for diabetic atherosclerosis	Replacement	Mette Bjerre University of Aarhus
Reducing the group size in studies of dermatitis by standardization of the gut microbiota	Reduction	Axel Kornerup Hansen University of Copenhagen
Refinement of animal models of pain: Establishment of strategies to alleviate avoidable pain in rat models for pain and inflammation	Refinement	Klas Abelson University of Copenhagen
Pathological and immunological consequences of basic experimental animal procedures in mice	Refinement	Dorte Bratbo Sørensen University of Copenhagen
2015		
An in vitro method to predict acute lung toxicity due to pulmonary surfactant inhibition	Replacement	Jorid Birkelund Sørli (tidl. Søren Thor Larsen) The National Research Center for Work Environment
A Refined Approach to Producing Polyclonal Antibodies in Chickens – Completely Replacing All Invasive Elements by Combining Immunizations with Routine Aerosol-based Vaccinations	Refinement	Otto Kalliokoski University of Copenhagen
An alternative to animal experiments: Development of an in vitro human skin model for evaluation of topical antimicrobial compounds	Replacement	Mette Elena Skindersø (June Lissa Hansen) Statens Seruminstitut

PROJECTS	R	RESEARCHER
2016		
Development of computer models to predict chemicals interference with thyroid hormones	Replacement	Marianne Dybdahl DTU-FOOD
Use of cell culture technology to minimize the needs for animal trials in development and production of fish vaccines	Reduction/ Refinement	Niels Lorenzen University of Aarhus
Towards better brain cancer treatment with novel in vitro models and fewer animal experiments	Replacement	Bjarne Winther Kristensen University of Southern Denmark
2017		
Use of Primary Isolates From Human Kidney to Study the Molecular Aspects of Blood Pressure Regulation	Replacement	Henrik Dimke University of Southern Denmark
Implementation of analgesic refinement in rats used as models for arthritis and inflammatory pain	Refinement	Klas Abelson University of Copenhagen
Developing FACS based in vitro assays to measure antibody mediated protection against infection with intracellular bacteria	Replacement	Jes Dietrich Statens Serum Institut
2018		
The mouse passport	Reduction	Axel Kornerup Hansen University of Copenhagen
New advanced blood infection model	Replacement	Thomas Emil Andersen University of Southern Denmark
Transport and metabolism of azol antifungal drugs in the human term placenta	Replacement	Bjarne Styrihave University of Copenhagen
2019		
Impact of shelter enrichment of metabolic cages in studies of protein metabolism	Refinement	Helle Nygaard Lærke University of Aarhus
Genetic modification of mice without the need for extensive breeding	Reduction	Per Svenningsen University of Southern Denmark
Investigation and analyses of internal validity in Danish preclinical research	Reduction	Birgitte S. Kousholt University of Aarhus

