

ANNUAL REPORT 2016

THE DANISH 3R-CENTER

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DEAR ALL

The chairman's foreword

The Danish 3R-Center started its work in late 2013 with a three-year grant. This means that, as the grant expired at the end of 2016, it is now a good time to review the centre's results.

We have made great accomplishments over these past three years with the establishment of a website providing lots of information and many news stories, holding annual symposia with inspiration and networking, distributing research funding, publishing newsletters, preparing teaching materials for both lower and upper-secondary schools, presenting 3R awards, setting up collaboration in Denmark and abroad, mapping 3R knowledge among users of laboratory animals – to mention just a few of our achievements.

In September 2016, the Danish 3R-Center organized yet another international symposium entitled "3R Successes". The many attendees and presentations served to prove that we definitely have many 3R success stories, which we could sometimes be better at spotlighting.

At the symposium, the 2016 3R Award was presented to the QSAR group at the National Food Institute. For more than 15 years, the research group has drawn on lessons learnt from previous animal testing, cell experiments and clinical trial data to develop mathematical models in their efforts to predict the effects of chemical substances in humans.

At the symposium, several of the research projects receiving funding from the 3R-Center were presented in talks or on posters. As was the case in 2014 and 2015, the 3R-Center awarded grants for three new research projects in 2016 and the centre has now supported a total of ten 3R projects.

For last year's annual report (2015), we asked Aarhus University to describe their enhanced focus on the 3Rs. This year, we have asked Novo Nordisk to present their

new 3R department to acknowledge and raise awareness of this effort. We would be happy to hear from other institutions that would like to describe their approach to making a difference in the 3R area.

Dissemination and communication is also an area of priority for the 3R-Center. As an example, last year's annual report gave an account of the preparation and publication of teaching materials on laboratory animals and the 3Rs for upper-secondary school students. In 2016, these teaching materials have been adjusted for use in the senior grades of the lower-secondary school (grades 8–10). The materials can be downloaded from our website (3rcenter.dk). We are generally continuing our efforts to develop and make the website more professional with information about laboratory animals and the 3Rs, as we take our obligations to disseminate information seriously.

Finally, I would like to take this opportunity to thank my fellow board members and the secretariat for their great dedication in 2016. It has been a pleasure working with you.

Last, but not least, I express my gratitude to the Danish Animal Welfare Society, LEO Pharma, Lundbeck and Novo Nordisk for their support for the Danish 3R-Center. Here, in late 2016, the decision was made to continue the Danish 3R-Center for three more years with the support from the above stakeholders; also, the animal welfare organization DOSO has opted to join the group of stakeholders. We welcome DOSO. With your support and that of the Ministry of Environment and Food of Denmark, we can continue creating a frontrunner environment for disseminating information and applying the 3Rs for the benefit of research and laboratory animals.

Christine Nellemann

Chairman of the board of the Danish 3R-Center

Laboratory animal centre to continue until 2020

Press Release, the Danish Veterinary and Food Administration, 20 December 2016

A handful of companies and lobby organizations have decided, together with the Ministry of Environment and Food of Denmark, to extend their support for the Danish 3R-Center, thus improving conditions for laboratory animals.

The Danish 3R-Center will continue to exist for another three years. This means that leading up to 2020, the centre will continue to support research into reducing the use of laboratory animals and improving their conditions. The stakeholders behind the Center are the Ministry of Environment and Food of Denmark, the Danish Animal Welfare Society, LEO Pharma, Lundbeck, Novo Nordisk and DOSO, the Cooperative Body of the Danish Animal Welfare Organizations. DOSO is a new partner.

“Having secured funding for the next three years, we are now able to continue our efforts to reduce the number of laboratory animals and further improve conditions for laboratory animals that still need to be used. This benefits both laboratory animals and the research,” says Christine Nellemann, Chairwoman of the board of the Danish 3R-Center.

Earlier this year, she and the other stakeholders behind the centre presented ten research projects launched by the 3R-Center since its inception in 2014.

“In just three years, the 3R-Center has achieved a great number of results which ultimately benefit laboratory animals. I’m particularly pleased that the 3R-Center has invested efforts in developing teaching materials for both upper-secondary school students and the senior grades of the lower-secondary school so that our future researchers can obtain knowledge of research animals based on factually correct information rather than myths,” says Per Henriksen, Chief Veterinary Officer, the Danish Veterinary and Food Administration.



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RESEARCH PROJECTS

An important aspect of the work carried out at the Danish 3R-Center is to initiate research projects that can improve laboratory animal issues.

RESEARCH GRANTS

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

Emphasis will be placed on the projects':

- Quality
- Implementability
- Relevance

Who is eligible to apply for funding?

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

Applications may be for up to DKK 500,000. The grants awarded typically amount to DKK 100,000–500,000.

Research funding for 2018

The round of applications for research funding for use in 2018 is expected to open in the autumn of 2017. Stay up to date at www.3rcenter.dk or sign up for our newsletter (www.en.3rcenter.dk/newsletter-subscription/) so that you do not miss the deadline.

A black and white photograph of a white rabbit sitting in a wire cage. The rabbit is looking towards the right. Its left ear is visible, and the number '2016' is printed on it. The cage has a metal grate, and there is hay in the background and foreground. The text 'PROJECTS SUPPORTED IN 2016' is overlaid on the image in a large, white, sans-serif font.

PROJECTS SUPPORTED IN 2016

In 2016, the 3R-Center received 14 applications for grants, of which the following three received a total of approx. DKK 1.5 million.

Towards improved treatment of **brain cancer** with novel cell-based models and less animal testing

Bjarne Winther Kristensen, University of Southern Denmark

The number of laboratory animals used in the development of new cancer medicines has been rapidly increasing in the past years. This also applies to brain cancer where human cancer cells are implanted directly in the brains of the laboratory animals. In cancer research, it is very important that the cancer cells in the models applied share the same characteristics as human cancer cells.

In the field of brain cancer, migrating cancer cells which spread in the brain and the so-called cancer stem cells that are believed to be the origin of the cancer are considered particularly important as they are the reason why this form of cancer is difficult to treat.

The project aims to investigate whether two new cell-based models can replace animal testing. In

one model, human cancer cells are implanted in brain tissue slices from mice. The issues investigated are how the cancer cells spread and the prevalence of cancer stem cells. The other model looks into how cancer cells spread on a lifelike surface in a culture medium that preserves the occurrence of cancer stem cells. These test results will be compared with results of tests carried out previously in which cancer cells were implanted in the brains of mice. This means that there will be no new animal testing.

The results of the project will be able to bring about less expensive and faster trials for novel cancer medicine, using new cell-based models. The results of the project will consequently benefit both animal welfare and cancer research.

PROJECT STATUS AT JANUARY 2017

The first series of testing with both models has shown how cancer cells spread rapidly, just as in the brain. The results also show that the stem-cell characteristics of the cancer cells are largely preserved, which would apparently make the models highly valuable research tools as they can help prevent animal testing to a greater degree.

The first set of results from the project was recently published in *Journal of Neurooncology* (2016, 130(1):53-62) and *PLOS ONE* (2016, 11(7) e159746). The next project phase will focus on whether the molecular mechanisms governing cancer-cell characteristics match the existing knowledge of cancer-cell migration. This would further strengthen the applicability of the models.

Developing **computer models** to predict the influence of chemicals on thyroid hormones

Marianne Dybdahl, Technical University of Denmark

Chemicals that might interfere with the body's endocrine systems, causing detrimental effects, have become a source of increasing worry. Until now, the prime focus has been on chemicals affecting the balance of sex hormones, but the effect on thyroid hormones is now receiving much attention.

In addition to their metabolic role, thyroid hormones also play an important part in the early development of the brain. Even moderate and transient reductions of the mother's hormone levels during pregnancy can adversely affect the development of the child's brain and nervous system.

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

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

This project aims to develop computer models for some of the many mechanisms by means of which chemicals can affect thyroid hormones. Among the methods that we will use to develop the models are experimental test data to which we have been given access through our collaboration with the US Environmental Protection Agency.

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

The predictions will be made available in a free and public online database. This virtual screening enables us to rapidly and cost-efficiently identify potential endocrine disruptors among the many chemicals in our food, environment and consumer products. The new models can also contribute to the future design of safer medicines and chemicals.



PROJECT STATUS AT JANUARY 2017

Two of the three QSAR models have now been developed and validated. The first model can predict whether chemicals activate a receptor with an effect on hormone turnover. This work has just been accepted for publication in *Computational Toxicology*¹. The other model can predict chemicals' inhibition of an enzyme that is instrumental in forming the hormones. We are currently typing up the results of this work for a scientific paper. The latter model is being developed and can be used to predict chemicals' activation of another receptor which is also involved in hormone conversion.

¹QSAR Development and Profiling of 72,524 REACH Substances for PXR Activation and CYP3A4 Induction. Rosenberg, S. A., Xia, M., Huang, R., Nikolov, N. G., Wedebye, E. B., and Dybdahl, M.

Using **cell cultivation techniques** to minimize the need for laboratory animals in the development and production of vaccines for farmed fish

Niels Lorenzen, Aarhus University

For both humans and farm animals, preventing infectious diseases is better than treating a disease once contracted. This also applies to fish farming where the increased use of vaccines over the past 30 years has resulted in a significant decline in the use of antibiotics. Developing vaccines for fish and checking the quality of vaccines already produced are largely based on vaccination and infection testing on laboratory animals.

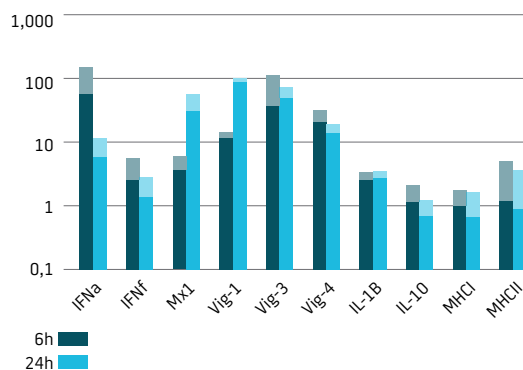
This project aims to develop protocols and methods for using cell cultures in the initial studies of how vaccine components are absorbed in and affect live cells – including any toxicity. The work will include both analysis of gene regulation and microscopic examination.

The project will be associated with other, ongoing research projects involving vaccination testing on fish, and the project will consequently not use any additional laboratory animals.

The results are expected to contribute to a partial replacement of the use of laboratory animals by cell cultures in the initial stages of the development and production of vaccines for fish. This will help reduce the need for laboratory animals in vaccine research and the vaccine industry and refine animal testing by reducing the level of distress as components with toxic side effects can be sorted out before testing on animals.

GENE EXPRESSION IN RTG-11 CELLS

polyIC-treated related to non-treated samples



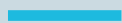
Q-PCR is a method for measuring modified gene activity in cells and tissue. The gene activity in treated cells is compared with the activity in untreated cells. As expected, the results show activity in a number of genes that play a role in the immune response. These results will be compared with previous results from fish testing.

PROJECT STATUS AT JANUARY 2017 7

A panel of cell lines has been selected for the purpose of representing different tissue in the fish, for instance cells cultivated from the liver, intestines or immune cells from rainbow trout. A number of genes of importance to the immune response have also been selected. The initial cell line testing has been performed.

Preliminary results: polyIC is a known immune response stimulator as the system recognizes it as a sign of virus infection and thus a hazard indication. The below figure shows RTG-11 cells (gonad cells) stimulated with polyIC. The gene expression of a series of genes has been examined using Q-PCR.

STATUS UPDATES FOR RESEARCH PROJECTS SUPPORTED BY THE DANISH 3R-CENTER IN **2014** AND **2015**



JANUARY 2017

‘Artificial blood vessels’ – a model for investigating diabetic arteriosclerosis / 2014

Mette Bjerre, Aarhus University

One in three diabetic patients develops cardiac diseases. The mortality rate of their disease is almost five times higher than in non-diabetics. The reasons have yet to be identified, but diabetics’ high blood sugar level is considered to be a significant factor in the harmful effect on blood vessels.

The research project aims to identify some of the mechanisms involved in arteriosclerosis in diabetics.

With the support of the Danish 3R-Center, we have developed a human cell model – a kind of artificial blood vessel – that mimics intercellular communication in the vessel wall. This affords us a unique opportunity to investigate the importance of blood sugar for the subsequent signalling between endothelial cells and smooth muscle cells. The model system also replaces the use of animal models in our mechanistic studies.

Our studies show that endothelial cells have different responses to varying glucose levels and this change is also reflected in the muscle cells’ migration ability. In addition, the metabolic memory of endothelial cells also appears to affect/inhibit the ability of the cells to adapt to a changeable environment.

We are currently investigating the significance of the glucose-dependent change in signalling and the subsequent difference in migration ability for the development of arteriosclerosis in diabetics.

Standardizing gut microbiota in mice as a tool for reducing the number of animals in the individual experiments / 2014

Axel Kornerup Hansen, University of Copenhagen

The main idea behind the project was that over the past 5–10 years, it has been demonstrated in a number of research areas that the more diverse the gut microbiota in the animals, the less homogenous their reaction is in tests and the greater the number of animals that are consequently required. For this reason, it can be assumed that if you either make the gut microbiota in the mice more homogenous or provide them with a gut microbiota that ensures a high response, you can reduce the number of mice to be tested.

One of the disease models showing the greatest correlation is a mouse model for children's eczema – also known as atopic dermatitis – which is why this model was chosen for this project. However, the issue is also relevant for other disease models such as models for diabetes, obesity and Crohn's disease. The

project has now been completed in its entirety and is awaiting final approval for publication.

Mice that are born germ-free were inoculated with the gut flora of mice with either a high or low susceptibility to children's eczema. The mice inoculated with bacteria from high-response donors exhibited a significantly higher clinical score, enlarged ear thickness and elevated levels of the important transmitter substances IL-1, TNF-alpha, IL-4, IL-5 and IL-6 compared with mice inoculated with bacteria from low-response donors.

The inter-individual variation was generally not affected by this increase in effect. Non-inoculated germ-free mice exhibited high, but also highly variable, disease activity, which suggests that the missing response in some mice is due to the protective characteristics of certain bacteria. Mathematically speaking, the higher effect may possibly reduce the group size, but it is important to conduct further studies – both to show that efficient treatment for testing of mice will still be able to reduce this increased disease level to the usual low level, which is necessary for reducing the group size, and to make an attempt at finding the protective bacteria so that mice with these bacteria can be sorted out before the test as they cannot contribute to the conclusion of a test, anyway.



PHOTO: The Danish 3R-Center

Refinement of animal models of pain: Developing methods to alleviate pain in laboratory-rat pain research / 2014

Klas Abelson, University of Copenhagen

This research project has aimed to examine how parameters of relevance in animal models for inflammatory diseases and pain research are actually impacted when the animals receive pain management. The hypothesis was that it would be possible, to a far greater extent than previously, to manage pain in rats used as models for inflammatory pain than is currently the case. This means that unnecessary pain inflicted on the animals can be restricted and thus minimized. This will optimize animal welfare and reduce stress and consequently reduce variation among the individual animals, thus increasing the reliability of the studies.

The project started in November 2014, and the experimental work was completed in October 2015. During this period, we have studied the effects of buprenorphine, which has no or only little effect on inflammation, in rats with induced arthritis in a hind leg joint (monoarthritis). The effects examined are parameters for animal welfare and relevant disease parameters. The effects were compared with animals without pain management and with animals treated with anti-inflammatory medicine.

The overall results are that pain management had no or little effect on clinical and pathological development of arthritis parameters. Caprofen, an anti-inflammatory substance, appeared to be better at inhibiting joint stiffness and swelling than the opiate buprenorphine, as expected. The difference between the groups concerning pain, stress and welfare was subtle. The most notable difference was reduced hyperalgesia in one of the buprenorphine groups.

This leads to the conclusion that based on our results, there is no immediate reason to restrict buprenorphine analgesia in rats used as an arthritis model in the model in question. More studies are required to draw more specific conclusions, however. There is always room for further improvement of the model and for identifying in more detail when and for how long the animals must receive pain management.

The results from the study are being compiled in a manuscript for publication in a scientific journal during the spring of 2017.

Pathological and immunological consequences of different blood sampling methods in mice / 2014

Dorte Bratbo Sørensen, University of Copenhagen

Millions of mice are used for testing on a global scale every year, and blood samples are taken from many of these mice. The choice of the best blood sampling method depends on the purpose of the test, as any impact on related parameters should be avoided, as well as on the degree of adverse effect on the mouse's welfare. For this reason, it is important to know the impact of the various blood sampling methods on animal welfare and the immune system and the amount of tissue damage caused.

In this study, we tested six different frequently used methods for drawing blood from mice and observed the animals at various times for up to twelve days after blood sampling. Each time, histopathological changes were assessed, both locally at the sampling site (including healing time) and systemically in selected organs (liver, kidneys, spleen and lungs). Systemic inflammation was assessed

by measuring haptoglobin in the blood and locally by determining the gene expression of inflammatory markers such as S100A8, S100A9 and Cxcl2, IL-6, IL-1b, IL-33 and Nlrp3. Animal welfare was assessed by measuring stress hormone levels, appetite and weight loss in the days following sampling.

All blood sampling methods caused changes, but to a varying degree. Animal welfare generally appeared to be most affected by sampling in the head region whereas one of the methods for blood sampling from the tail generally resulted in the greatest impact on inflammatory parameters. This means that it is not possible to unambiguously identify "the best method," but careful consideration should be given to which method is best suited for the test in question.



PHOTO: The Danish 3R-Center

Developing an *in vitro* method to predict acute pulmonary toxicity from aerosol proofing products / 2015

Jorid Birkelund Sørli, The National Research Centre for the Working Environment

Aerosol products for weatherproofing shoes, textiles, furniture and construction materials are used both by private individuals and in professional settings. During application of the products, small droplets of fluid will form in the air (aerosols) that can be inhaled. Some products have caused acute toxic poisoning of persons during the spraying process. The typical symptoms are coughing and shortness of breath but the effects can be more serious, including reduced pulmonary function.

The current standard method involves the use of laboratory animals and is associated with discomfort and some suffering for the animals. We have shown that proofing sprays are toxic, primarily because they inhibit the function of a pulmonary surfactant which is vital for the function of the lungs. When the pulmonary surfactant is functioning, breathing is effortless. When toxic proofing aerosols are deeply inhaled into the lungs and react with the pulmonary surfactant layer, the surfactant's function is destroyed. As a result, the pulmonary alveoli begin to collapse. The opening and collapse of the alveoli explains the intoxication symptoms experienced by users shortly after application of toxic products.

In this project, we have tested 22 products in a dynamic model of the pulmonary surfactant function – an artificial alveolus. The surface of the pulmonary surfactant is increased and reduced with the same frequency and extent as in the lungs, and at the same time the surfactant is exposed to the proofing spray product. The products were divided into two groups – toxic and non-toxic – based on whether the surfactant function was inhibited or not. To test the usefulness of the *in vitro* method, the same products were tested with a modified *in vivo* model. The modified method minimizes the discomfort and suffering of the animals, for instance by stopping the exposure before the mice become very affected by the products. There is a good correlation between the *in vitro* and *in vivo* methods. In 77% of the cases, the *in vitro* model predicts the result of toxicity for the mice. Using the *in vitro* method can reduce the necessity of using laboratory animals to test the toxicity of proofing spray products, even before marketing, and thus prevent the poisoning of users during application.

A Refined Approach to Producing Polyclonal Antibodies in Chickens – Completely Replacing All Invasive Elements by Combining Immunizations with Routine Aerosol-based Vaccinations / 2015

Otto Kalliokoski, University of Copenhagen

The goal of our project is to immunize chickens for antibody production without ever having to touch or restrain them. By attaching the immunogen (the antibody target) onto the virus particles of an inhalable vaccine which all chickens in Denmark is given after birth, we believe that we can provide immunity to the disease (infectious bronchitis) while simultaneously producing antibodies. We have previously developed methods for extracting antibodies from hens' eggs and within the project we have refined our methods for detecting immunity toward the vaccine based on the hen's antibodies. In addition we have confirmed that we can successfully immunize

chickens with quantities of immunogen small enough to be carried on the surface of virus particles and developed a method for combining the two (and methods for evaluating the conjugation process). Finally, we have developed a method for delivering a microliter-scale aerosol to day-old chickens without having to restrain them.

Looking back on the previous year, our project has come a long way, but one unforeseen problem has prevented us from putting all the elements of the project together for a final test. Chicken vaccines are not developed to be nearly as pure as humane vaccines. Using electron microscopy we found that the commercial vaccines contained more – by several orders of magnitude – impurities and stabilizing ingredients than they contained actual attenuated virus. These impurities interfere with the conjugation methods we have developed, effectively preventing us from attaching the immunogen to the virus. We are therefore currently working on a method for purifying the virus particles from the commercial vaccine preparation in a gentle enough manner to not damage its virulence. We are currently holding off on carrying out a final experiment on chickens before we believe that we have a satisfactory purity of the starting material and can test our hypothesis under optimal conditions.



PHOTO: The Danish 3R-Center

Artificial skin in a Petri dish as an alternative to laboratory animals / 2015

Mette Elena Skindersø, Statens Serum Institut

There is a great need for new antimicrobials that can be used to fight skin infection, such as that caused by Methicillin-Resistant Staphylococcus Aureus (MRSA). A skin infection model in mice is often used in this context. This project will examine whether skin cultivated in a Petri dish can be used as an alternative/supplement to the animal model, both for testing such new substances and for examining pathogenic characteristics of different bacteria strains.

The project will use data from previously performed skin infection experiments in mice as its reference. This means that the project will not involve new animal testing.

The artificial skin that is being used in the project is based on donated human skin cells, for instance from people who have had excess skin removed after obesity surgery.

We have just carried out a Proof of Concept test in which we have made wounds in the artificial skin and infected them with MRSA bacteria and then examined the bacterial count in the following days. We have also treated some of the skin pieces with cream that either contains fucidin or mupirocin – both antimicrobials used for skin infection. Data from this experiment show that the bacteria are highly capable of infecting the artificial skin and that the treatment efficiently reduces the amount of bacteria in the artificial skin to a degree that corresponds well to similar testing of skin infection in mice.

The experiment will now be repeated to confirm data, and other strains of Staphylococcus aureus will be tested to examine whether their infection potential corresponds to the MRSA strain already used.

2



DISSEMINATION

In 2016, we were keenly focused on providing information to students at both lower and upper-secondary school level.

There are two reasons in particular for this focus: First, the young people represent the coming scientists and potential users of laboratory animals and we can present the 3R concept to them already now. Second, it is our impression that the knowledge of many young people (and adults for that matter) on laboratory animals is not necessarily based on facts, as much of the information that you see online, for instance, is either untrue or highly opinionated.

For this reason, we have taken a series of initiatives to enhance knowledge of the 3Rs among school students and took the first steps towards building a scientific knowledge bank on our website where the information is based on reliable sources only.

Website 3rcenter.dk

Our website is the most important platform in our efforts to communicate knowledge of laboratory animals to people who do not have a professional interest in the field of laboratory animals. Our partnership with the Danish Animal Experiments Directorate enables us to publish all statistics concerning laboratory animals that we find relevant for school students learning about laboratory animals, for instance. This means that school students can quickly and readily find information on our website about the animal species used for testing in Denmark, the number of animals used, etc.

In addition, we have started publishing easy-to-grasp texts on laboratory animals in general as well as on specific species of laboratory animals. So far, we have uploaded small texts on mice, rats, pigs and horses to the website but more will be added regularly.

We are aware that the information on the website should not only be of a high quality and credible. It must also be accessible if we want to disseminate knowledge about the subject. The vast majority of school students and others without a professional interest in working with laboratory animals are not familiar with the Danish 3R-Center. This means that if they search for information about laboratory animals, they will not access the Danish 3R-Center's website

directly in most cases. Against this background, we work continuously to optimize the material in internet search engines (primarily Google), thus considerably increasing their chances of finding our material when searching for information about laboratory animals.

Of course, the website is also a resource for people in the laboratory animal and alternatives segment who can find information about research projects, the symposium, etc. But the above scientific knowledge bank on the website on laboratory animals also includes scientific publications which are naturally more aimed at this group than at school students and the similar.

So far, we have placed eight laboratory animal-related articles on the website – Animal models for psychiatric diseases (Gregers Wegener), Pigs help save lives (Benedict Kjærgaard), Practical pain management in laboratory animals (Klas Abelson et al.), Courses in animal testing improve research outcomes and animal welfare (Aage Kristian Olsen Alstrup), You wouldn't do testing on a horse, would you? (Julie Fjeldborg), The pig as an animal model for human diseases (Henrik Callesen), Fish as laboratory animals – fish can also be used for testing, but there is a method to the madness (Kurt Buchmann) and Testing on exotic animals (Tobias Wang).

Teaching materials for the upper-secondary school

As is known, the Danish 3R-Center had teaching materials on laboratory animals and the 3Rs developed for use in upper-secondary school.

Just like the information on our website, the material is only of value if there are upper-secondary school classes that use the material, which is why we make an effort to raise awareness about it. In addition to arranging for links to the teaching materials to be placed at relevant websites (EMU Denmark's Learning Portal, Astra – National centre for learning in nature, technology and health in Denmark, among others), we have held two presentation day events about the material.

Therefore, on 3 March 2016, the 3R-Center invited interested upper-secondary school teachers to a presentation of the material in Aarhus. Teachers from seven upper-secondary schools attended the presentation day at which Tom Bengtsen of the Danish 3R-Center started by talking about laboratory animals and the 3Rs, followed by a presentation of the teaching materials by its developer, Aiko Sho Nielsen. Finally, the 3R-Center had invited Birgitte Kousholt of Aarhus University to talk about laboratory animals in practice.

The teaching materials were developed for Biology and Biotech classes, but the attending upper-secondary school teachers informed Aiko about the interdisciplinary qualities of the subject. The teachers at the event mentioned that it was difficult to find subjects for their cross-disciplinary tasks that could combine humanistic or social-science disciplines with science subjects, but they could definitely see potential, for instance in the area of ethics. The Danish 3R-Center will attempt to incorporate these considerations into any supplementary materials, if realized.

On 25 November 2016, the teaching materials were presented at Sukkertoppen Upper-Secondary School in Valby to eight upper-secondary school teachers attending the event, all from schools in Greater Copenhagen. They heard Aiko Sho Nielsen and Tom Bengtsen talk about the material, laboratory animals and the 3Rs and were introduced to the quiz in the teaching materials.

Several among the teachers said that they would use the materials in their respective classes in the near future.

It is also worth noting that teachers will not necessarily use the materials by following the individual steps rigorously but that they will rather apply a more individual approach. As an example, the secretariat heard from a teacher who only used the quizzes in the materials for first-year students. Another teacher related the following:

"The students answer quiz 1 as a home assignment and then read the background article. Based on the background article, they prepare five questions in groups. The next lesson is a quiz session where they answer the other groups' questions. Then they are given the second part of the quiz in the material. Next, I will show them a short film about August Krogh and discuss the contributions of animal testing with them. To show them an alternative to animal testing, I will read the article on lungs in your resource space, if there's enough time."

Teaching materials for lower-secondary school, grades 8–10

On 19 December, the Danish 3R-Center published teaching materials on its website for use in biology and biotech classes in the senior grades of lower-secondary school (grades 8–10). The materials were developed by schoolteacher Amanda Jespersen and are based on the 3R-Center's teaching materials for upper-secondary school. This means that the materials have been adjusted so that the degree of difficulty now suits grades 8-10. The materials consist of an introductory quiz to be taken before starting the learning segment, a background article with tasks to be solved during the process, a resource space with links to be used for group work and a final quiz which shows the teacher whether the students' attitude to animal testing has changed and whether they have acquired knowledge of the subject.

Amanda on the teaching materials: *"You can use the materials for coherent mini-segments of, say, 2–3 modules that will provide the students with a scientific background for considering the biological and ethical issues concerning the use of laboratory animals."*

The Danish 3R-Center will work towards having the materials presented at relevant teaching sites and we are also considering advertising in key professional journals, etc., to raise awareness of the materials.

Science Calling

The dissemination of information about laboratory animals and 3R received a substantial boost by Peter Bollen, member of the board of the 3R-Center, who visited no less than eight schools (Vejle, Horsens, Kolding, Gesten, Ullerslev, Samsø, Agerbæk and Esbjerg) as part of Science Calling (*Videnskaben på besøg*), meaning that around 700 students heard the talk. Science Calling is a lecture scheme under which scientists, students and others working with science, technology and health visit schools to tell their own science story.

In 2016, Science Calling was held in the week from 26 to 30 September. Peter Bollen visited the eight schools to give a talk that was presented as follows by the organizers of Science Calling (Astra):

“Animal testing is often considered a necessary evil. When push comes to shove, we all want access to treatment for diseases, and disease research often uses animal testing. But how

many laboratory animals do we actually use in Denmark and what are they used for? Why do we use laboratory animals in the first place? Do the animals suffer and are there alternatives to animal testing? This talk provides insight into the use of animals for research in Denmark and explains the legal and ethical boundaries of animal testing.”

Among the elements of the talk was a film from a laboratory animal facility, and the talk also included time for the students to ask Peter questions. The schools also evaluated Peter’s talk subsequently.

They all praised the lecture, and Peter also saw the school visits as a positive experience. The Danish 3R-Center has therefore already promised Astra to contribute in 2017 because we also wish to give high priority to dissemination of knowledge about laboratory animals to students.

“Well communicated. Integrated quizzes along the way and good contact with the students”

“Kept at a level that the students understood, and Peter listened to the students when they had questions”



3

EVENTS

To give an impression of national and international 3R developments, this section present some of the events that we have either attended or organized.

World Day for Laboratory Animals

29. april

For the fifth consecutive year in Denmark, the Danish Animal Welfare Society (DAWS) and DOSO organized the World Day for Laboratory Animals that highlights current topics and issues related to the use of laboratory animals. This year was themed around *“Replacement/Reduction – the Danish effort for replacement and reduction in animal testing”*.

DAWS and DOSO had planned an exciting and versatile programme, opened by Esben Lunde Larsen, Danish Minister for Environment and Food, who talked about why laboratory animals are necessary in drug development and also emphasized the importance of continuously seeking improvements in the laboratory-animal area; a viewpoint that is well in sync with the Ministry of Environment and Food Denmark’s support for the Danish 3R-Center.

The programme not only included talks on research-specific replacement and reduction, but also talks on ethics, replacement and reduction in relation to REACH (basic EU chemicals regulation aimed at ensuring the prudent use of chemicals with minimum risk to health and environment), the Danish Animal Experiments Directorate’s coordinating work with respect to animal welfare bodies, the 3R efforts by Novo Nordisk as well as best practice and alternatives in an education perspective.

Like last year (2015), the Danish 3R-Center was part of the programme as our board chairwoman, Christine Nellemann, gave a presentation entitled: *“The Danish 3R-Center: annual status with particular focus on replacement/reduction initiatives,”* in which she talked about the seven replacement/reduction projects that have received grants from the Danish 3R-Center during its three years of existence.

One of these seven projects was presented at the event – An alternative method for examining

chemicals for acute pulmonary toxic effects, presented by Søren Thor Larsen of The National Research Centre for the Working Environment. The project aims to map the extent to which the acute toxic effect of proofing spray products can be predicted using an *in vitro* method.

Søren Thor Larsen told the audience that preliminary data suggests that the model is excellent at predicting which spray products are toxic for mice to inhale and which are harmless. This means that the model holds obvious potential for toxicology screening of spray products, for instance, and that it can potentially reduce the need for laboratory animals in this area (read more about the project in the appendix and see the project status in section 1).



Annual meeting of the animal welfare bodies

3. juni 2016

Each year, the Committee for Laboratory Animals and Alternatives organizes a meeting for animal welfare bodies to discuss the function of these bodies.

The meetings have successfully been expanded so that the programme for the animal welfare bodies takes place before noon, and the nature of the meeting changes in the afternoon to become an inspiration event offering networking for everyone with a professional interest in laboratory animals.

This year's meeting was held on 3 June 2016 at Lundbeck in Valby with representatives from no less than 37 of Denmark's 45 animal welfare bodies. First, they learned about the work undertaken by the Committee for Laboratory Animals and Alternatives since last year's meeting, for instance the drawing up of a series of guidelines concerning standardized procedures and effects that are often included in animal testing applications. The following four are available from 1 January 2017: Guidelines for blood sampling from mice and rats; Guidelines for weight loss in mice and rats; Guidelines for fasting of mice and rats in experiments; Guidelines for substance dosages for mice and rats in experiments (the guidelines are available at the Danish Animal Experiments Directorate's website: dyreforsogstilsynet.dk).

This was followed by a talk by Peter Sandøe (University of Copenhagen) on ethical dilemmas, before Kirsten Bayer Andersen of the Committee for Laboratory Animals and Alternatives/the Danish 3R-Center gave a talk on legislative requirements of animal welfare bodies.

Next on the programme was a workshop on the interrelation between legislative requirements and ethical dilemmas on the one hand and the work of animal welfare bodies on the other. The workshop ended with a plenary session at which many interesting aspects and ideas were discussed and shared.

After lunch, the meeting was opened to everyone with an interest in the subject, kicking off with three talks on the 3Rs by an animal keeper (Panum), a scientist (DTU Aqua) and a manager (Zealand Pharma A/S) who shared the lessons they had learned in their respective positions at an animal facility.

The last part of the programme with networking and inspiration has developed into a great success. It not only attracts a lot of interested participants; many also bring and display 3R initiatives which have had a positive effect on animal testing in their respective institution/company in one way or the other. The initiatives are put on display and people move between the stands to learn about the objects and ideas shown which have either improved animal welfare, refined a method, brought about a reduction of the number of laboratory animals, etc.

As a source of inspiration, you can visit the Committee's website to which these initiatives are uploaded (go to www.fvst.dk and search for the Committee's Danish name: "Udvalget for Forsøgsdyr og Alternativer").

Note: This year (2017), the annual meeting of the animal welfare bodies will be held on 4 April.

THE COMMITTEE FOR LABORATORY ANIMALS AND ALTERNATIVES

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

The Committee members are also members of the board of the Danish 3R-Center.

ANIMAL WELFARE BODY

The animal welfare body is tasked with the following:

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

FELASA – Federation for Laboratory Animal Science Associations

13–16 June, Brussels

The FELASA Congress is held every third year in Europe. The conference is keenly focused on the design of cages and facilities, technical equipment, animal models, rules for keeping and using laboratory animals, etc.

It is evident that meetings and conferences on the use of laboratory animals see an increasing number of contributions on the 3Rs and the work to refine, reduce and replace laboratory animals. This was also the case at last year's FELASA Congress with no less than 1,750 participants. Entire sessions were reserved for contributions on new 3R knowledge, and animal welfare organizations, researchers, the European Commission, companies and professional associations all presented talks on the great efforts to promote the 3Rs undertaken at global level.

Tom Bengtson, Head of Secretariat at the Danish 3R-Center, gave a presentation on the centre's work during its almost three years of existence. Our teaching materials received much attention, and other, similar organizations were very interested in finding inspiration in them. The centre's survey of laboratory-animal users' knowledge and use of 3R methods also aroused interest.

At the same session, Norecopa, NC3Rs and EPAA (European Partnership for Alternative Approaches to Animal Testing) each gave a presentation on their initiatives. Particularly

impressive was the presentation of Norecopa's new combined and modernized database of 3R resources.

The survey of laboratory-animal users' knowledge and use of 3R methods was published at the conference. It was presented by Jesper Lassen of the University of Copenhagen who carried out the survey on behalf of the Danish 3R-Center (read more about the project in the section on the Danish 3R-Center's symposium).

One observation of particular note from the conference was that four workshops on assessing the suffering of laboratory animals, held by David Anderson, a representative of the European Commission, were remarkably popular. In fact, pre-booked admission tickets for the workshops became a popular bartering item among the conference participants! The Danish 3R-Center is looking into the possibility of inviting David Anderson to Denmark in 2017 to enable Danish scientists to acquire this knowledge.

The overwhelming interest in the workshop clearly shows the great desire among users of laboratory animals to get the right tools for assessing the discomfort that the animals are exposed to, thus becoming able to improve their conditions.

The next FELASA Congress will be held in Prague in 2019.



PHOTO: Sønderby & Grathwohl | Fotograf

EEMGS Annual Meeting 14-18 August

EEMGS (European Environmental Mutagenesis and Genomics Society) and ECOPA (European Consensus Platform for Alternatives) were behind the meeting, which was organized by a committee chaired by Lisbeth E. Knudsen, member of the board of the Danish 3R-Center. A total of 10 sessions and 53 posters provided the backdrop for highly rewarding scientific discussions themed around bridging the gaps between genomics, risk assessment of human exposure to environmental impacts and the 3Rs in animal testing. A total of 180 participants from 18 countries (Belgium, the Czech Republic, Finland, France, the Netherlands, Italy, Japan, South Korea, Norway, Russia, Slovakia, Sweden, Switzerland, Germany, UK, USA and Austria) and many young participants ensured a broad spectrum and lively discussions in breaks and during poster sessions.

The 3R-Center sponsored a one-day event dedicated to the latest trends in 3R and genotoxicity at which the latest OECD guideline

on data sharing was presented, and the good experience of *in vitro* methods for genotoxicity were emphasized. The 3R-Center also sponsored the participation of three young scientists who presented their results with posters and short talks: Sine Rosenberg of the National Food Institute with the title: *Development of a QSAR model for thyroperoxidase inhibition and screening of 72,526 REACH substances*; Jenny Aasa of Stockholm University with *Exclusive genotoxicity data for glycidol to be used for cancer risk estimation*; and Alexandra Opalinska of the University of Copenhagen with *Transfer of paracetamol, aniline and the metabolites in the human placental transfer model*.

The journal *Basic Clinical Pharmacology and Toxicology* was the main sponsor and will publish a special issue with select presentations. A number of other institutions and companies sponsored the rent of premises, food and printing of the programme – including Novo Nordisk and Lundbeck.

EUSAAT – European Society for Alternatives to Animal Testing 24-27 August

Once again, the Danish 3R-Center was represented at the congress in Linz. Its name notwithstanding, the congress has developed into a congress for all 3Rs – probably as a consequence of the fact that complete replacement of all laboratory animals is not realistic in the near future despite progress in the field of alternatives.

The congress attracted around 500 participants from more than 50 countries, which could definitely be seen as an indication that the 3Rs have made headway, also outside the EU. For instance, at a session on global cooperation and implementation of the 3Rs, a Japanese presenter (Hajime Kojima, National Institute of Health Sciences) told about the Asian Congress 2016 on Alternatives and Animal Use in the Life Sciences which is the first conference of its kind for scientists from Asia (mainly China, Japan, South Korea and India). The conference provides an opportunity to promote alternative methods among scientists in countries where the 3R concept is only just gaining a foothold (the conference was held on 15–18 November 2016).

A topic of interest to the Danish 3R-Center was the contribution by Elliot Lilley of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) who gave a presentation on his organization's particular efforts to focus on severe suffering among laboratory animals: "*Focus on severe suffering: an update on RSPCA activity to end severe suffering.*" There were multiple reasons for this focus: First, the ethical prudence of reducing suffering; second, the legal obligations based on EU Directive 2010/63/EU; third the scientific advantages based on the notion that high scientific quality is well underpinned by good animal welfare.

Even though only few animals in Denmark experience the most severe suffering (0.89% corresponding to 2,168 animals (2015)), the Danish 3R-Center, like the RSPCA, has decided to give this group of animals the centre's attention in order to examine whether it is possible to reduce the number of the most stressful procedures and thus reduce the number of animals in the highest category of suffering.

In this connection, we can find inspiration in many of the initiatives launched by the RSPCA in this important area, involving a number of online resources and publications. In June 2016, they also organized an international meeting entitled *Focus on severe suffering*, with no less than 150 delegates from 24 different countries (see www.rspca.org.uk/severesuffering).

At the conference in Linz, education was also a major theme, with two ninety-minute sessions being dedicated to this subject. The presentations in this session all pivoted on academia and research. It would appear that the Danish 3R-Center's development of teaching materials for lower and upper-secondary school students is quite unique and as awareness of our materials grows, it will be interesting to see if other 3R centres or similar organizations will be inspired by our efforts to enhance knowledge of laboratory animals and the 3R concept among young people.

Adrian Smith (secretary of Norecopa and member of the board of the Danish 3R-Center) gave an interesting presentation on Norecopa's new website (norecopa.no), which was developed with a view to increasing accessibility to (visibility of) species-specific guidelines in animal testing.

The website integrates several databases in one and a new search engine has been developed which can retrieve content from both databases and websites.

In connection with a session on the implementation of the EU Directive, Birgitte Kousholt of Aarhus University gave a presentation entitled *Establishing a culture*

of care by implementing AUGUST – a new and strong initiative at Aarhus University. The presentation visualized the university's great effort to implement the 3Rs, among other things by enhancing the contact of the animal welfare body with the university management and create a consistent approach down through the organization to reach scientific staff, veterinarians, animal care technicians and animal keepers.

Birgitte Kousholt talked about how Systematic Reviews and Meta Analyses in particular are becoming central topics at the university, which is not just an advantage in terms of 3R but also enhances scientific quality at the university.

The Danish 3R-Center presented a poster promoting our website, the ten 3R projects we have supported financially and our annual international symposium and other activities.

We definitely find it relevant to be present at the conference in Linz and will certainly consider participating in 2018 (the conference is not organized in 2017 due to the 10th World Congress on Alternatives and Animals in the Life Sciences (WC10), which will be held on 20–24 August 2017 in Seattle, Washington, USA).



The Danish 3R-Center's symposium

13–14 September 2016

Our international symposium is an important event in our efforts to promote awareness and dissemination of the 3Rs. The symposium presents current research and legislation to the participants, and the event provides a good opportunity to meet peers and colleagues.

Themed on 3R success stories, this year's symposium was held in Copenhagen with no less than 180 attendees with an interest in laboratory animals and alternatives in one form or another (researchers, students, animal care technicians, animal keepers, animal welfare organization representatives, etc.).

2016 PROGRAMME

The year's conference was chaired by Axel Kornerup Hansen who is a board member of the Danish 3R-Center. Axel opened the symposium with his thoughts about the great turnout which he considered an indication of the symposium's relevance.

Axel then moved on to present the year's keynote speaker, author and science journalist Lone Frank.

ANIMALS – ARE THEY ALMOST PEOPLE?

Lone Frank, author and science journalist

In her talk, Lone Frank used research into the cognitive and emotional life of various animal species as her point of departure. Surprisingly, this research reveals sophistication in animals where we might not expect it, such as in rats which appear to be capable of being empathetic towards each other. Lone Frank wanted to point out that such realizations should have a bearing on the laboratory animals, as it should spark more debate on the extent to which the individual animal tests are really necessary.

THE DANISH 3R-CENTER – SUCCESSES SO FAR

Christine Nellemann, the Danish 3R-Center

Christine Nellemann provided a status update on the 3R-Center's work during its first three years of existence, accounting for the many initiatives that the Center has made to promote the 3Rs in Denmark.

With respect to the website, Christine told about the ideas underlying the structure of the site. The 3R-Center is not just tasked with communicating relevant knowledge

to people with a professional interest in laboratory animals and alternatives, but it also has tasks aimed at educating the public, which the website should take into account, of course. The website's news in both Danish and English targets the former group, and this is also the case for the scientific papers on laboratory animals and the information on research projects receiving support from the centre, whereas teaching materials and facts on laboratory animals and animal testing have a general educational purpose (read more about the website in section 2).

Christine moved on to talk about the ten 3R projects that have received financial support from the centre. Christine was happy that the quality of the applications received had increased over the years and she was pleased with the promising results of several of the supported projects.

Last, but not least, Christine Nellemann viewed the higher attendance at the year's symposium as an indication that awareness of the centre is growing, which is definitely a success story as it results in greater knowledge of the 3Rs among participants and colleagues which ultimately translates into a positive effect for laboratory animals.

LABORATORY ANIMALS – PERSPECTIVES FROM AN ANIMAL WELFARE ORGANIZATION

Kirsten Rosenmaj Jacobsen, the Danish Animal Welfare Society

Kirsten Rosenmaj Jacobsen opened her presentation with facts about the Danish Animal Welfare Society such as employee resources (86 full-time equivalents), number of volunteers (approx. 1,000) and membership (75,000 members).

Before touching on laboratory animals in her presentation, Kirsten talked about the animals' homes and the Animal Emergency Centre that you can call if an animal is in distress.

When it comes to laboratory animals, the Danish Animal Welfare Society works actively for the replacement of laboratory animals with alternatives. Until all animals have been replaced with alternatives, the Society works to ensure the best possible welfare for the animals that cannot yet be replaced by alternatives. This means that attempts should be made to minimize the number of laboratory animals, and the animals should be ensured the best possible conditions in terms of housing, care, etc.

The Danish Animal Welfare Society actively worked to set up the Danish 3R-Center back in 2013 and subsequently played an important role in bringing together animal welfare organizations, the pharmaceutical industry and the government in their joint interest in promoting the 3Rs.

In addition, Kirsten told the symposium that the Danish Animal Welfare Society also endeavours to inform and influence the general public in matters of laboratory animals and animal testing through campaigns, seminars, etc.

IS IT POSSIBLE TO REDUCE GROUP SIZE OR INCREASE POWER BY USING MICE WITH HIGH RESPONDING MICROBIOTA IN STUDIES OF DERMATITIS?

Line Sidsel Fisker Zachariassen, University of Copenhagen

This presentation is not included here, as a project description is included in the appendix and the project's status is found in the first section of this annual report.

SUCCESSES, CURRENT ACTIVITIES AND FUTURE PLANS OF THE GERMAN CENTRE FOR THE PROTECTION OF LABORATORY ANIMALS (BF3R)

Barbara Grune, BfR

The first international presenter at the symposium was Barbara Grune of Germany's Federal Institute for Risk Assessment (BfR; *Bundesinstitut für Risikobewertung*), who started by talking about the establishment of the German Centre for the Protection of Laboratory Animals (Bf3R) in September 2015 which aims to promote the 3Rs at national level, like the Danish 3R-Center. All of the centre's activities aim to minimize the number of laboratory animals and guarantee the best possible protection of laboratory animals.

Barbara then went on to talk about a new initiative in Germany, based on Article 43 of Directive 2010/63/EU, which will provide the general public with reliable, transparent data on animal testing to inform discussions in this field. Towards this end, the Animal Test Info database publishes easy-to-grasp and non-technical (anonymized) project summaries from approved animal testing in Germany, which makes the database a unique platform for scientists to communicate directly to the general public (www.animaltestinfo.de).

Barbara Grune also gave an account of some of BfR's areas of expertise, including alternative methods in toxicology and the reduction of stress levels and animal welfare improvements. The former includes development of new *in vitro* test methods, for instance, while the latter includes R&D into methods for reducing pain and suffering in laboratory animals (refinement).

TOWARDS BETTER BRAIN CANCER TREATMENT WITH NOVEL CELL-BASED MODELS AND FEWER ANIMAL EXPERIMENTS

Bjarne Winther Kristensen, University of Southern Denmark

The presentation is not included here, as the project is described above in this annual report (section 1).

RECENT HIGHLIGHTS FROM EURL ECVAM

Susanne Belz, EURL-ECVAM

Susanne Belz opened her presentation with a brief introduction to EURL ECVAM which is the EU reference laboratory for alternatives to animal testing, before she went on to talk about some of the results they had achieved.

Some of the more significant results are found in the field of risk assessment of chemicals. EURL ECVAM has thus embraced the concept of the mechanistic approach in its risk assessment of chemicals, facilitated by the Adverse Outcome Pathway concept. This means that a lot of laboratory animals can be spared in investigations of the possible adverse effects of chemicals (potentially carcinogens, allergens, reproductive damage, etc.) as attempts are made at predicting the danger of the chemical using previously gathered knowledge of chemicals. A specific example of this is the acceptance of valid animal-free models in sensitivity testing of chemicals under REACH (the EU chemicals legislation).

EURL ECVAM also works to promote the use of alternatives, for instance through their databases and search guides that offer tools for stakeholders to obtain comprehensive knowledge of alternatives (see: <https://eurl-ecvam.jrc.ec.europa.eu/databases>).

REFINEMENT OF ANIMAL MODELS OF PAIN: ESTABLISHMENT OF STRATEGIES TO ALLEVIATE AVOIDABLE PAIN IN RAT MODELS FOR PAIN AND INFLAMMATION

Klas Abelson, University of Copenhagen

This presentation is not included here, as a project description is included in the appendix and the project's status is found in the first section of this annual report.

EPAA PROGRESS AND SUCCESSES IN 3RS FOR BIOLOGICALS

Catrina Stirling, European Partnership for Alternative Approaches to Animal Testing

Catrina Stirling opened her presentation with a brief introduction to the European Partnership for Alternative Approaches to Animal Testing (EPAA) which is a voluntary partnership between the European Commission and industry stakeholders. The partners will collect knowledge and resources to advance the development, validation and approval of alternatives to animal testing in tests required by law. Seven industrial sectors have made a commitment to support alternative methods (Animal Health, Chemicals, Cosmetics, Crop Protection, Fragrances, Pharmaceuticals (including vaccines) and Soaps & Detergents).

An interesting theme in Catrinas presentation was about the EPAA's Vaccine Consistency Approach Project – a project launched in 2010 to eventually achieve considerable reductions of the number of animal testing in connection with quality control of human and veterinary vaccines. EPAA started the project for the purpose of overcoming the remaining scientific and technical challenges relating to the quality control of vaccines without the use of laboratory animals, and they also attempted to promote an animal-free approach in the legislation. The project has definitely raised awareness of an animal-free approach among stakeholders in the area, and the project has produced promising results in connection with human rabies vaccines and clostridium septicum.

(If interested, you can read more about the project here (peer reviewed article): De Mattia et al 2015, The Vaccines Consistency Approach Project: an EPAA initiative, *Pharmeuropa Bio & SN*, May 2015, pp. 30–56.)

Symposium 13-14 September 2016

PROGRAMME

13 September

9.00-10.00	Registration and breakfast	-
10.00-10.10	Welcome by moderator	Axel Kornerup Hansen The Danish 3R-Center
10.10-10.55	Animals – are they almost people?	Lone Frank Author & Science journalist
10.55-11.05	The Danish 3R-Center – successes so far	Christine Nellemann The Danish 3R-Center
11.05-11.20	Break	-
11.20-11.45	Laboratory animals: perspectives from an animal welfare organization	Kirsten Rosenmaj Jacobsen The Danish Animal Welfare Society
11.45-12.00	Is it possible to reduce group size or increase power by using mice with high responding microbiota in studies of dermatitis?	Line Sidsel Fisker Zachariassen University of Copenhagen
12.00-13.00	Lunch	-
13.00-13.40	Successes, current activities and future plans of the German Centre for the Protection of Laboratory Animals (Bf3R)	Barbara Grune BfR
13.40-13.55	Towards better brain cancer treatment with novel <i>in vitro</i> models and fewer animal experiments	Bjarne Winther Kristensen University of Southern Denmark
13.55-14.40	Recent Highlights from EURL ECVAM	Susanne Belz (EURL-ECVAM)
14.40-15.00	Break	-
15.00-15.15	Refinement of animal models of pain: Establishment of strategies to alleviate avoidable pain in rat models for pain and inflammation	Klas Abelson University of Copenhagen
15.15-16.00	EPAA progress and successes in 3Rs for biologicals	Catrina Stirling European Partnership for Alternative Approaches to Animal Testing
16.00-18.00	Poster session with wine, cheese and networking	-

14 September

08.30-09.00	Breakfast	-
09.00-09.30	The OECD framework for Adverse Outcome Pathways Development and Application	Magda Sachana OECD
09.30-09.50	The Danish 3R Survey – Knowledge, attitudes and experiences with the 3Rs among researchers involved in animal experiments in Denmark	Jesper Lassen University of Copenhagen
09.50-10.15	CRACK IT Solutions: successes from the NC3Rs technology partnering hub	Joanna Edwards NC3R
10.15-10.45	Break	-
10.45-10.55	Introducing the 3R prize winner	Lisbeth E. Knudsen The Danish 3R-Center
10.55-11.05	3R – prize winner	QSAR-team
11.05-11.50	Preparing dogs for study life: improving welfare, efficiency and data output	Laura Hall University of Sterling, NC3R Prize Winner
11.50-12.35	The RSPCA and the 3Rs: the scientific animal welfare organisation as a catalyst for change	Elliot Lilley RSPCA
12.35-12.50	Pathological and immunological consequences of different blood samplings procedures in mice	Dorte Bratbo Sørensen University of Copenhagen
12.50-13.00	Concluding remarks	Axel Kornerup Hansen
13.00-15.00	Lunch and networking	-

THE OECD FRAMEWORK FOR ADVERSE OUTCOME PATHWAYS DEVELOPMENT AND APPLICATION

Magda Sachana, OECD

The first talk of the second day of the symposium was given by Magda Sachana, from the OECD. She started out by describing how the traditional method of testing chemicals in OECD countries uses animal testing to study any problematic effects of the chemicals on human beings or the environment. For almost 35 years, the OECD has harmonized these *in vivo* testing methods and established testing guidelines, which are internationally recognized standards, along with the OECD's principles of good laboratory practice.

At a global level, many laboratory animals are used for such testing (although not many in Denmark), a large number of which are enormously resource-intensive in terms of both the number of laboratory animals and time required. According to the OECD, a total of 5,000 experimental animals are needed to test one chemical, and the time required can extend up to several years with testing prices ranging from EUR 2,000 to EUR 2,000,000. Seeing that there are around 100,000 chemicals on the market, tests based on animal testing are not possible for resource reasons, which is why only 10–20% have been studied. For reasons of animal welfare and economic viability, it is necessary to develop methods that enable the testing of chemicals within a much shorter time frame.

The OECD has already standardized some animal-free testing methods for the testing of a wide range of toxicological effects, such as testing for skin and eye corrosion, irritation, phototoxicity, genotoxicity and hormonal disturbances. For instance, it is possible to test corrosiveness and irritation by using *in vitro* methods based on human skin models which imitate the upper layers of human skin.

THE DANISH 3R SURVEY – KNOWLEDGE, ATTITUDES AND EXPERIENCES WITH THE 3RS AMONG RESEARCHERS INVOLVED IN ANIMAL EXPERIMENTS IN DENMARK

Jesper Lassen, University of Copenhagen

The Danish 3R-Center had launched a Danish 3R study (2016) for which Jesper Lassen was responsible and which was presented at this year's symposium.

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

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

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

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

When asked directly about the barriers to further implementation of the 3Rs, many emphasized the lack of relevant research and innovation as a significant factor. They also singled out increased data sharing and a higher degree of cooperation among researchers as factors that could promote the implementation of reduction.

Similarly, scientific factors, such as better accessibility of human tissue, a greater number of relevant cell cultures and better computer models were identified as the most important conditions for further replacement.

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

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

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

CRACK IT SOLUTIONS: SUCCESSES FROM THE NC3RS TECHNOLOGY PARTNERING HUB

Joanna Edwards, NC3Rs

The next talk was given by Joanna Edwards from NC3Rs, an organization established by the British government in 2004 on the basis of the 3Rs to support science, innovation and animal welfare, with an annual budget of around GBP 10 million.

Important elements in NC3Rs' work are CRACK IT Challenges and CRACK IT Solutions. The first-mentioned aims to help the industry find solutions to specific challenges in such a way as to get 3R-related benefits. The last-mentioned is aimed at drawing on NC3Rs' vast network in the academic world and industry to promote already existing research and technology with 3R potential for the purpose of identifying partners who can boost research/technology to a higher level.

Joanna presented several success stories from the CRACK IT Solution's programme, such as one relating to the development of pharmaceuticals. Renal toxicity is a frequent cause of drugs being rejected by drug-approval authorities. One scientist (Dr Colin Brown, Newcastle University) had developed an *in vitro* renal proximal tubule assay for drug safety testing, a human cell model which he hoped could be used by the industry to screen substances to provide an early indication of nephrotoxicity (damage to kidneys caused by exposure to a chemical). Initially, Dr Brown wanted to find a partner with whom he could cooperate to gain access to compounds with nephrotoxic properties that he could then use to validate his model. Next, he wanted to engage in cooperation that could improve the model's complexity.

The CRACK IT Solutions programme resulted in Dr Colin Brown landing an agreement with SOLVO Biotechnology, which now provides the screening method to their customers. As a result, the technology is now used by most pharmaceutical companies, enabling them to reduce the number of laboratory animals, as well as refrain from further testing of compounds that are very likely to damage kidneys.

Presentation of the Danish 3R-Center's 2016 3R award

The next item on the agenda was the presentation of the year's awards. The 2016 3R award went to the QSAR team at the National Food Institute, Technical University of Denmark. For more than fifteen years, the team has been making a big effort to provide a powerful Danish contribution to the area "*computer-based predictions of the dangerous properties of chemicals*".

The team's contribution was worded in the nomination as follows:

Their efforts have contributed to and been consequential for:

- the OECD project involving the proliferation of the use of QSAR in regulatory efforts and the industry. In this context, a comprehensive and freely accessible software system – the OECD QSAR Toolbox – has been developed. Eva Bay Wedebye and Nikolai Georgiev Nikolov have contributed and still contribute to both its development and content. Since then the toolbox has been used by a great many researchers and companies all over the world, including consulting firms in their efforts to advise the pharmaceutical industry:
- Providing advice to the Danish Environmental Protection Agency as part of predicting the dangerous properties of many chemicals, of which knowledge is lacking, and prioritizing the required action to be taken for these chemicals.
- Establishment and scientific development of the National Food Institute's spin-off company – Saxocon – concerning candidate drugs and contamination of drugs (www.saxocon.com).

Going forward, their efforts will contribute to the following:

- Development of integrated testing strategies for chemicals and drugs that will constitute a new paradigm for assessing the dangerous properties of chemicals and drugs.
- Development of safer chemical substances to substitute hazardous substances, as the computer models can initially screen out many candidates due to undesirable effects.
- The development of QSAR models for important mechanisms identified in the OECD'S "*Adverse Outcome Pathways*" (AOP), which can be used to predict undesirable effects. This could provide a more targeted design of animal testing in which better and more relevant information is obtained.
- Assessing the hazardousness of substances contained in cosmetics, as it is forbidden to use laboratory animals for this.

The team's main performers are currently Eva Bay Wedebye and Nikolai Georgiev Nikolov, whose efforts are internationally recognized. Their actions have been crucial for reducing the use of laboratory animals to test chemicals and pharmaceuticals, which is one of the most important reasons why the board of the Danish 3R-Center decided that this year's 3R award should go to the QSAR team.

THE 3R AWARD

Each year, the Danish 3R-Center gives an award to a person or group of persons affiliated with a company, university or other body who work to promote the 3Rs in Denmark. The award is presented as part of the annual symposium. In the period before the symposium, nominations can be sent to the Danish 3R-Center, after which the board decides who should receive the award, which comes with a diploma and DKK 10,000.



PHOTO: Sønderby & Grathwohl Fotograf

PREPARING DOGS FOR STUDY LIFE: IMPROVING WELFARE, EFFICIENCY AND DATA OUTPUT

Laura Hall, University of Sterling

Laura Hall based her talk on the fact that even though many dogs are used as laboratory animals at a global level (68 dogs were used in Denmark in 2015), we have only a limited amount of knowledge of how the routine actions relating to the experiments affect the dogs' welfare.

She presented a number of techniques for preparing dogs for life as a laboratory animal, techniques that could provide various improvements, such as better handling of dogs, reduced risk of injury to dogs and staff, greater staff job satisfaction, better animal welfare, etc.

As with a wide range of other refinement initiatives, it is also necessary to assess the effects of the training programme by using a validated method, which is why the research group has developed an animal welfare monitoring tool for use by animal keepers, etc., to monitor the training effects.

Laura also presented the website [http:// www.refiningdogcare.com](http://www.refiningdogcare.com), offering various resources to people involved with laboratory dogs in one way or another.

THE RSPCA AND THE 3RS: THE SCIENTIFIC ANIMAL WELFARE ORGANISATION AS A CATALYST FOR CHANGE

Elliot Lilley, RSPCA

Elliot Lilley works in the laboratory animal department at the Royal Society for the Prevention of Cruelty to Animals (RSPCA). The department has defined a number of key fields of work that are intended to pave the way for their principle goal of replacing laboratory animals with humane alternatives.

The fields of work are as follows: Challenge the use of animals on ethical and scientific grounds; ensure an efficient and well-enforced regulation of animal testing; boost standards at international level; promote an open, candid debate; promote animal welfare for educational and training purposes at advanced study programmes; and reduce the use of laboratory animals and their suffering.

For a number of years, the department has been involved in setting up expert working groups to discuss best practices and formulate guidelines for a wider scientific environment. The expert working groups have usually included researchers from academic environments and the industry, as well as veterinarians and animal care technicians – all of whom possess expertise in the given topic.

The RSPCA recently formed an expert working group focused on implementing the 3Rs in research areas where experiments which cause significant discomfort to animals can occur. Elliot Lilley explained how they had already published guidelines concerning the refinement of epilepsy models, experimental autoimmune encephalomyelitis, rheumatoid arthritis and sepsis. As previously mentioned in this annual report, animal testing which causes significant discomfort will also be monitored by the board of the Danish 3R-Center, to look into the possibility of replacing, reducing or refining these experiments.

Some of the interesting initiatives discussed by Eliot relate to the above-mentioned field of boosting animal welfare standards internationally, i.e. the RSCPA's training events for public officials, members of animal ethics committees, researchers and animal keepers, events, which the organization has held in countries such as Bulgaria, Croatia, China and Taiwan, as well as the material produced by the RSPCA on animal well-being improvements in many

different languages. As mentioned in the description of the EUSAAT conference in Linz, the 3R concept is budding forth in places like China, which is why countries and organizations with longer-standing traditions in the 3R field can play an important role in further disseminating the concept, not only nationally but also internationally.

PATHOLOGICAL AND IMMUNOLOGICAL CONSEQUENCES OF DIFFERENT BLOOD SAMPLINGS PROCEDURES IN MICE

Dorte Bratbo Sørensen, University of Copenhagen

The presentation is not included here, as a project description is included in the appendix and the project's status is found in the first chapter of this annual report.

CONCLUDING REMARKS

Axel Kornerup Hansen, The Danish 3R-Center

Axel expressed his gratitude to both the speakers and the many participants.

NB: This year's symposium (2017) will be held at the same venue on 7–8 November.

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The program was varied and interesting. While it is unlikely that I personally will attend the next symposium, I have feedback to colleagues that it was an interesting and relevant meeting and an NC3Rs representative may well attend next symposium in 2017.



Satisfaction survey about the symposium

After having carried out a satisfaction survey among the symposium's participants, we noted that the respondents were generally quite satisfied with the event. More than half of the participants took time to respond to the 3R-Center's questionnaire, where each participant was asked to rate their level of satisfaction (or dissatisfaction) on a scale of 1 (dissatisfied) to 5 (very satisfied), which resulted in an average rating of 4.1.

Many participants chose to give us some input about next year's symposium. Several people indicated a wish for slightly longer breaks, and many felt that some of the talks were too long. The Danish 3R-Center will definitely be guided by this input to make next year's symposium even better.

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There could have been even more presentations about specific 3R-related studies, including data presentations. Some of the EU-related presentations were a little longer. The event could have made do with one or two of these presentations per day, lasting 35 minutes each, for instance. The poster session with wine and snacks was brilliant.

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I think that the programme is dynamic by combining current research with 3R working methods from abroad. I thought that the coffee breaks were a little too short though. Networking is a big part of these events and there were several I wanted to speak with that I didn't have time for. But I know that you have to strike a balance with the breaks.

4



3R ACTIVITIES AT NOVO NORDISK

On the lines of last year's annual report, where the Danish 3R-Center gave the floor to Aarhus University to hear about the 3R efforts taking place there, this year we are giving Novo Nordisk the opportunity to tell about their efforts in the 3R area. In our view, many people are curious about the company's creation of an in-house 3R department, which gives the impression that the company, similar to Aarhus University, takes its 3R efforts seriously.

3R STRATEGY & VETERINARY SERVICES – THE 3R DEPARTMENT AT NOVO NORDISK A/S

Stine Øvlisen

Director of 3R Strategy & Veterinary Services

In 2015, Novo Nordisk decided to implement a more strategic approach to the company's 3R activities by creating a centrally located 3R department (3R Strategy & Veterinary Services).



The department comprises a team of six employees who in the aggregate have vast experience in the fields of research, veterinary science, laboratory animals and the three Rs. The department is centrally located at the heart of research and is led by the department manager for Laboratory Animal Science (LAS), which also includes the animal housing units. This achieves an important integration of the department, in terms of both research and the practical work being done in the housing units. The objectives of the 3R department are put together so they seek to meet elements of the company's bioethics strategy. The department endeavours to have projects big and small in progress at the same time; similarly, the department should be engaged in ongoing activities within all 3R fields, all aimed at achieving the best possible scientific progress with the best animal welfare possible.

The department works together with R&D staff to create 3R innovation focused on animal welfare, animal ethics and improvements in the use of laboratory animals, which boost research at the same time. The department serves as a discussion partner and provides feedback to departments working on animal testing and running activities identified within the 3Rs. All activities aim to promote animal welfare, which includes to reduce or replace the use of animals, and to help get better scientific data so that patients can ultimately be given the best treatment possible.

REPLACEMENT

One area of focus of the department is to replace the use of live animals. Over time, quite significant replacement results have been achieved at Novo Nordisk, which include eliminating the use of live animals for quality control of marketed products.

A lesson learned from this work was that it requires a dedicated, targeted effort, as well as the efforts of many stakeholders, to succeed at this. Therefore, the department aims to be a driving force in terms of seeking out replacement options in-house, prioritizing and maturing project proposals, applying for resources and, in cooperation with the employees at the involved departments, launching projects aimed at reducing and eventually replacing the use of live animals.

To launch this task, the department held a interdisciplinary workshop for employees cutting across their fields of expertise and research for the purpose of generating the first ideas for potential replacement initiatives. This workshop resulted in the drafting of a catalogue of ideas which describes all possible – and (currently) impossible – replacement and reduction proposals. Based on this, work groups will be formed and project proposals will be submitted.

REDUCTION

As part of identifying possible projects to replace the use of live animals, a number of initiatives with the potential to reduce the number of live animals were also identified. These ideas will be evaluated and matured, and work groups will also be formed and project proposals will also be submitted as described above.

Continuous efforts are being made in the individual research departments and project groups to improve methodologies which lead to reduction and better science. This is exemplified by the implementation of micro-sampling and thus the requisite modification of assays and the re-use of animals wherever possible from an animal-welfare perspective.

REFINEMENT

Novo Nordisk's global standards are based on Danish and European legislation, but attitudes and best practice can vary depending on national conditions and legislation.

To ensure that the entire organization observes the company's global standards, Novo Nordisk implements an in-house training programme at all of the company's animal housing units for employees involved with animals;

similarly an exchange programme has been implemented for employees from the various sites. The Animal Welfare body that approves all experiments conducted under the auspices of Novo Nordisk serves as an overarching steering committee for local issuers of permits (IACUC). This ensures a common approach and common standards. For example, a project has been launched across the company's global research units aimed at preparing best-practice guidelines for the taking of blood samples based on relevant experiment set-ups.

In a large, global undertaking, colleagues, project managers and sister departments can be quite far apart. This can complicate the knowledge-sharing process and exacerbate the risk that good initiatives are not disseminated to all relevant parties quickly enough. To mitigate this risk, the sharing of methodological improvements and refinements must be gathered and ensured. In this context, a model catalogue has been created so that everyone across departments and global sites can adhere to the same procedures and use the same methods. Specific work groups have been created to collect knowledge and improvements concerning some of the most widely used models, which gives us the best, most refined models. For example, one work group is working to collect all in-house improvements and lessons learned in induced diabetic models to ensure that the gentlest, most reproducible model is used, and that it is composed of all the valuable initiatives launched by employees across the company worldwide.

To guarantee that the 3R mindset is included in the scientific and business-related discussions that are conducted concerning research projects and project plans, a representative from the 3R department now attends all meetings where this issue is discussed.

CROSS-CUTTING INITIATIVES WITH PARTNERS

Novo Nordisk conducts the vast majority of its animal testing in-house, but a small portion is carried out by external partners. Some of the important steps towards improving the conditions for our animals have been taken in these collaborative efforts. We frequently experience that we and our partners are a source of inspiration for one another and that, through the problems we encounter along the way or by challenging one another, we can launch initiatives that lead to improvements for the animals. Together with a contract research organization (CRO), we launched a project to improve housing conditions as part of regulatory experiments with dogs, and, as the first project milestone, we are now able to replace experiments where dogs had to be included in the experiments alone with experiments where dogs can be used in pairs during the experiment. This has strikingly improved the dogs' welfare.

STUDENT PROJECTS

Some of the tasks that need to be carried out, including problems that need to be illuminated to address the 3Rs, will take place in the form of shorter, temporary projects. Educational institutions are essential partners in these projects, as a number of projects can be set up as student assignments, theses, doctoral dissertations and post-doc positions. This type of cooperation has been initiated and more will be launched on an ongoing basis as required.

EDUCATION, TRAINING, KNOWLEDGE-SHARING AND INFORMATION

A vital part of efforts involving ongoing improvements in terms of the 3Rs are in the form of further education, knowledge-sharing and information. To systematize this, we have created LAS Academia, which, in addition to providing the already established courses, will also be providing a vastly greater number of courses and workshops targeting the needs found in research. User meetings will be held on an ongoing basis to share important information and news about the latest initiatives. This is exemplified by our regular dialogue meetings with permit-holders.

External knowledge-sharing is also an important part of the department's tasks. It is important that we, as an undertaking, share the good initiatives we develop so that animals elsewhere can also benefit from them. We can do this by participating in conferences, writing articles and giving lectures, and we can also invite interested parties inside. As a department, it has been possible for us to support the employees of the research departments in getting 3R initiatives drafted and submitted to conferences, which they would otherwise not have participated in, or to have articles published. We bring in external knowledge on our own initiative or based on requests from colleagues. In order to share knowledge, the department staff are actively involved in various external forums, networks and societies/groups. The 3R department should serve as a "single point of entry" and a representative of Novo Nordisk in animal-ethics and animal-welfare contexts.

The department is off to a good start and it has already proven to be an effective way to help a research organization – that has high ambitions, good intentions and dedicated employees – on its way towards even more initiatives to replace, reduce and refine the use of the animals that are needed in the research and development of drugs to improve the quality of life of people suffering from severe chronic illnesses.

5



INTERNATIONAL COOPERATION

This year (2016, the Danish 3R-Center continued to bring some of its focus to bear on international partners, as the relatively modest size of our centre, compared to the UK's NC3Rs for instance, makes the establishment of a cooperation network a logical step towards disseminating information about the 3Rs. For this reason, we have taken part in various meetings in both Nordic and European frameworks, and we have also set up an agreement concerning NC3Rs' dissemination of 3R material to all of the country's experiment venues.

In 2016 we also received the good news that Sweden is establishing a 3R centre in 2017, with substantial budgetary funding into the bargain. At the Danish 3R-Center, we are convinced that this centre will be an excellent partner for us going forward. On top of that, perhaps the Swedes can even be inspired by us in establishing their 3R centre, as we have gradually become a well-established and well-run centre.

STRENGTHENED 3R CENTRE FOR BETTER PROTECTION OF ANIMALS

Government Offices of Sweden, 16 September 2016

In its 2017 Finance Bill, the Government recommends allocating SEK 15 million annually from 2017 to 2020 to a centre of expertise to deal with 3R issues. The aim of the undertaking is to strengthen the protection of animals through coordination and skills development in the area of animal testing. The centre is also intended to support animal-testing ethics boards in issues concerning alternative experiment methods.

The area comprising alternatives to animal testing and 3R issues (replace, reduce and refine) is broad and comprises a wide variety of undertakings with numerous players involved. In order to improve efforts involving alternative methods to animal testing, the lessons learnt and the expertise of the parties involved – such as authorities, universities, colleges, research funders and organizations – must be utilized and efficient cooperation among them must be developed. The Swedish Board of Agriculture's

recently founded 3R centre is charged with coordinating 3R issues in Sweden.

“Sweden is a pioneering country in terms of animal protection but there is always room for improvement. This is why it seems natural and expedient that we can now get this undertaking up and running in earnest to minimize the need for animal testing,” says Sven-Erik Bucht, Sweden's Minister for Agriculture.

The funds which are proposed to the Swedish Parliament will be important to guarantee the responsible use of laboratory animals according to the 3R principles. The strengthening not only underpins strong protection of laboratory animals, but also contributes to the development of research. The 3R centre is also an important player in the implementation of the European Directive on the protection of animals used for scientific purposes.

FUTURE TASKS OF THE 3R CENTRE:

- Coordinate and develop cooperation among various players.
- Ensure that the expertise and experience of the involved parties are utilized.
- Support animal-testing ethics boards in issues concerning alternatives to animal testing.
- Collect, process and disseminate information about alternative methods.
- Assist in the preparation of government research allocations targeting this area.

3R STANDS FOR:

- **Replacement:** to replace animal testing wherever possible with other methods that do not include animals.
- **Reduction:** to make it possible for researchers to get sufficient data using fewer animals.
- **Refinement:** to refine methods to minimize any pain and discomfort in the laboratory animal.

The proposal in the 2017 Finance Bill is based on an agreement between the government parties and the Left Party.

Meeting between European 3R centres and the European Commission

27–28 October, Glostrup, Denmark

In late October 2016, the Danish 3R-Center was visited by a delegation from the Norwegian Food Safety Authority, which is the Norwegian counterpart of the Danish Veterinary and Food Administration. The Norwegian Food Safety Authority is responsible for issuing permits to carry out animal testing in Norway, as well as conducting inspections. Because it is not a member of the European Union, Norway is not under an obligation to implement the EU directive, but the directive is included in the agreement with the EEA and is implemented in Norway. (The European Economic Area is based on an agreement between the EU and Norway, Iceland and Liechtenstein which makes it possible for the three countries to be part of the EU's Single Market.)

For this reason, the delegation was very interested to hear how Denmark is addressing the implementation and any challenges arising from this.

They were also very interested in efforts by the Danish 3R-Center and the Committee for Laboratory Animals and Alternatives to strengthen research, knowledge-sharing and implementation of 3R initiatives. The Norwegians were impressed by the many projects we have launched. We are maintaining contact going forward for the benefit of 3R developments in both countries.

Meeting with the Norwegian Food Safety Authority

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Seminar: **How European Union Reference Centres for Animal Welfare could be organized,** 1 December, Uppsala, Sweden

In cooperation with the Animal Welfare Knowledge Centre (Denmark), the Swedish Centre for Animal Welfare (SCAW), the Finnish Centre for Animal Welfare, the Norwegian Veterinary Institute and the Lithuanian Veterinary Academy, the Danish 3R-Center is also actively participating in NordCAW (Nordic network for Communicating Animal Welfare), which works to strengthen cooperation between Nordic countries and the Baltic States concerning the communication of animal-welfare issues.

For the second consecutive year, NordCAW organized a seminar in Uppsala, which this year (2016) was based on the EU's intentions to establish an information platform concerning animal welfare to promote dialogue and to share knowledge between stakeholders, and the possible revision of Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed

and food law, animal health and animal welfare rules. The revision contains two articles about the establishment of a number of reference centres for animal welfare and the spheres of responsibility and tasks of these centres.

In this light, the following question, among others, was discussed: How will we get the stakeholders (farmers, industry, retailers, veterinarians, animal scientists, animal welfare organizations, consumers organizations etc.) connected, as a part, a reference group? The discussion clarified that the various stakeholders' respective agendas can make it difficult to closely cooperate in terms of a reference centre.

The Danish 3R-Center still considers its contacts with the other NordCAW partners to be valuable in efforts to disseminate knowledge of animal welfare/laboratory animal welfare in the Nordic countries and the Baltic States, which is why we are continuing to maintain this connection.

Improve your research: distribution of material from NC3Rs

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) in the UK has prepared a number of resources that can support research before, during and after experiments are carried out. These resources range from tools which can support the planning of study design (Experimental Design Assistant (EDA)), help determine and score end points (grimace scale posters for mice, rats and rabbits), ARRIVE guidelines, which are important for a correct and complete publication, as well as CRACK-IT, which is a platform for disseminating information about 3R-related research.

The Danish 3R-Center finds the material to be particularly relevant, which is why we chose to distribute the materials and raise awareness

about them among researchers, animal keepers and others who work in Denmark's animal housing units on a daily basis. As a result, we have sent the materials to all animal welfare bodies in Denmark, and anyone who is interested can order the materials on our website (3rcenter.dk/forskning/forbedr-din-forskning). It is our hope that the NC3Rs' grimace-scale posters will end up hanging everywhere in Denmark where animal testing is being carried out, to increase staff awareness of the animals' well-being.

Based on the NC3Rs materials we have also prepared an advertising poster aimed at raising awareness of the materials among researchers to increase the likelihood that they will use tools such as EDA and the ARRIVE guidelines in their research.

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 testing and create even better conditions for laboratory animals.

PER HENRIKSEN, CHIEF VETERINARY OFFICER, THE DANISH VETERINARY AND FOOD ADMINISTRATION

“I have followed the efforts of the Danish 3R-Center over the past three years with great interest – efforts that have resulted in a series of results that will ultimately benefit laboratory animals. The Ministry of Environment and Food of Denmark has consequently decided to extend its support for the Danish 3R-Center.”

THE DANISH ANIMAL WELFARE SOCIETY

“The Danish Animal Welfare Society (DAWS) believes that the use of laboratory animals should be minimized. DAWS therefore 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 THE DANISH ANIMAL WELFARE ORGANIZATIONS (DOSO)

“The overarching aim of DOSO is to abolish animal testing. Towards this end, DOSO is actively engaged in promoting the development, validation and implementation of alternatives to animal testing based on the 3R principle. 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.”