

ANNUAL REPORT 2015

THE DANISH 3R-CENTER

FOREWORD

2015 was a rewarding year for the Danish 3R-Center. We created a forum for our collaboration on and discussion of the 3Rs, with our highly successful symposium on 10–11 November 2015 as one of the highlights. The symposium included contributions from international partners, the research community and the European Commission.

We have launched a new and improved website that offers a good overview of the world of the 3Rs. We have also started issuing newsletters – for instance in relation to the distribution of research funding and in connection with the symposium.

In 2015, the 3R-Center launched three new 3R research projects that you can read more about in this annual report. We have followed up the four projects which we supported in 2014 – all of which have the potential to optimise laboratory-animal aspects within their respective fields of research.

In 2015, we have also had educational materials prepared for use in Danish upper-secondary programmes. The materials are available at no charge from our website where teacher manuals and other items may be found. We hope that these materials will be put to extensive use.

We have launched a project that analyses data on the use of laboratory animals in Denmark for the past 10 years in order to improve the basis for monitoring developments in the use of laboratory animals in Denmark.

We also initiated a major questionnaire survey about the awareness of 3Rs among users of laboratory animals in Denmark, since enhanced knowledge in this area may give us a better idea of how to introduce the 3R mindset among those who carry out experiments.

The Danish 3R-Center continues to focus on collaboration with international 3R organisations. In 2015, we arranged EU-level meetings with non-Danish 3R centres to improve our knowledge of the different areas of focus.

I would like to take this opportunity to thank the board and the secretariat for their commitment to the work of the Center.

Last, but not least, I would like to thank the Danish Animal Welfare Society, LEO Pharma, Lundbeck, Novo Nordisk and the Danish Ministry of Environment and Food for supporting the Danish 3R-Center, enabling the establishment of a leading environment for dissemination and application of the 3Rs for the benefit of both laboratory animals and research.

Christine Nellemann, Chairman of the Board of the Danish 3R-Center



CONTENTS

1 Research projects	04
→ Artificial skin in a Petri dish as an alternative to laboratory animals	05
→ Developing an <i>in vitro</i> method to predict acute pulmonary toxicity from aerosol proofing products	06
→ Can chickens be immunised with an aerosol combined with vaccination?	08
→ Research projects 2014	09
2 Website	10
→ Teaching materials	12
→ Press coverage of the Danish 3R-Center	13
3 3R events	14
→ World Day for Laboratory Animals	15
→ The Danish 3R-Center's symposium	16
→ Symposium and workshop about Systematic Reviews and Meta-Analyses	25
→ Networking and dialogue about the function of animal-welfare organisations	28
4 International cooperation	30
→ Finnish Centre for Alternative Methods (FICAM)	32
→ European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM)	33
→ European Society for Alternatives to Animal Testing (EUSAAT)	34
→ Nordic platform for Communication on Animal Welfare (NordCAW)	35
→ EU	35
5 Appendix	36
→ The Danish 3R-Center	37
→ Board	38
→ Secretariat	40
→ The 3Rs – Replacement, Reduction and Refinement	41



1 RESEARCH PROJECTS 2015

An important part of the Danish 3R-Center's activities is to launch research projects that can result in improvements in the field of animal testing. In 2015, the 3R-Center received 21 applications for grants and awarded the following three projects a total of DKK 1.3 million.

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

Mette Elena Skindersø, Statens Serum Institut

The research group wishes to halve the number of mice used for skin infection research by establishing an alternative to animal testing.

In Denmark alone, at least 500 mice are used every year for skin infection studies, which corresponds to approximately 25,000 mice at the EU level. It is expected that artificial skin cultivated in a Petri dish can be used for testing new drugs against skin infections caused by resistant staphylococci such as Methicillin-Resistant *Staphylococcus Aureus* (MRSA).

The group will also examine whether artificial skin can replace animal testing in comparisons of pathogenic characteristics of different bacteria strains in order to apply the method as broadly as possible.

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

The project will use data from skin infection experiments previously performed in animals as their reference, so the project group will not carry out new animal tests.

In addition to the ethical problems related to the use of laboratory animals, it is also expensive and difficult to carry out experiments in animals. The option of replacing some animal testing with a cell-based alternative will therefore both lower the cost and make it easier to test new antibacterial substances, for instance. In addition, the artificial skin can be cultivated using comparatively simple laboratory equipment, allowing also laboratories without animal-testing facilities to carry out infection studies.

Having such an alternative will consequently benefit both animal welfare and research into novel medicines.

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

Søren Thor Larsen, The National Research Centre for the Working Environment

Aerosol products for weatherproofing items such as shoes, textiles and construction materials are commonly used in private households and for industrial purposes. The products are designed to form a water- and dirt-repellent coat on the surface of materials.

During application, small droplets of fluid (aerosols) will form in the air and can be inhaled. Unfortunately, a small number of the marketed products have been shown to cause acute toxic poisoning of the user and other persons standing close by during the spraying process. Typical symptoms are coughing and shortness of breath, but they can also cause reduced pulmonary function and partial collapse of the lungs in the most severe cases.

The current standard method for examining aerosol products for acute pulmonary toxicity is to expose mice or rats to inhalation of aerosols from the product and measure the animals' respiratory function.

The research group has demonstrated that the toxicity of weatherproofing aerosols may be related to a breakdown of the so-called pulmonary surfactant, which is a thin fluid film covering the inside of the lungs. Pulmonary

surfactant prevents the lungs from collapsing during respiration. The fact that some proofing products can destroy the effect of the pulmonary surfactant is one of the reasons why partial lung collapse occurs during severe poisoning with these products.

By testing the effect of a number of aerosol products on pulmonary surfactant isolated from pigs, the research group established that the products that cause acute lung toxicity reactions in mice also destroy pulmonary surfactant in an *in vitro* model.

Unfortunately, the existing *in vitro* method only works on water-soluble products. This means that it has not been possible to test the many oil-soluble products that are also on the market.

The objective of the new project is to refine the *in vitro* method to enable the testing of a broader range of aerosol proofing products, including oil-soluble products. If the method proves viable, it can be used for screening new aerosol products for acute pulmonary toxicity and thus reduce the number of laboratory animals used for these purposes.



Artificial skin to replace laboratory animals

Ritzau, 10 February 2015

We must reduce the use of laboratory animals in pharmaceutical manufacturing. This Tuesday, Dan Jørgensen (Social Democratic Party), Danish Minister for Food, will therefore allocate grants of DKK 1.3 million to three research projects that will identify alternatives to animal testing.

"There are currently no viable alternatives to all the animal testing that is being carried out to develop essential human medicines. As a consequence, we must constantly look for new alternatives in order to reduce the use of live animals", says Dan Jørgensen in a press release.

One project will create a lung model to be used for testing chemicals that are currently being tested on animals. The other project will develop artificial skin for use in experiments involving agents to combat skin infection. The third project involves replacing of goats with chickens in the production of antibodies.

Chicken eggs contain large volumes of antibodies which means that is not necessary to draw blood from the chicken to obtain the antibodies, as is the case with goats.

"The three projects are very interesting and will be used to conduct research into damage to human lungs and MRSA skin infections, for instance. I hope that the projects are successful", Dan Jørgensen says.

Can chickens be immunised with an aerosol combined with vaccination? Study of a non-invasive method for producing antibodies

Otto Kalliokoski, University of Copenhagen

Large numbers of laboratory animals are used on a global scale for production rather than testing. The production of polyclonal antibodies is a billion-dollar industry that uses the immune system in mice, rats, goats and other mammals to produce sera for many applications, including the diagnosis of disease. The animals are injected several times in the process, for instance with disease-relevant proteins and with an adjuvant (a compound boosting the immune response), after which blood samples are taken and the antibodies are isolated.

Polyclonal antibodies perform a significant role in modern medicine and medical research and are very beneficial to society. However, the production process could be improved in terms of animal welfare. If antiserum is produced in chickens, no blood needs to be drawn. This is because a chicken egg is full of antibodies, as the egg is evolutionarily adapted to protect the potential offspring by utilising the chicken's immune system. The isolation of antibodies from eggs can therefore eliminate the blood-withdrawal process from the antibody production process (an egg provides far more antibodies than a blood sample from a rabbit, for instance). The only invasive procedure that remains is the locally irritating adjuvant injections.

The research group will examine whether the production of polyclonal antibodies can be made completely non-invasive if performed in chickens.

The chickens that lay the eggs we eat are regularly vaccinated against a number of diseases. Vaccines against respiratory diseases are often administered as aerosols which the chickens inhale. The attenuated virus that makes up the vaccine is still able to enter the chicken's body through the mucous membranes of the airways where it gets in contact with the immune-response cells that quickly build immunity to the virus in question.

The research group wants to utilise this process by connecting a protein to these virus particles. They hope to "kill two birds with one stone" by having the proteins accompany the virus into the chicken's body: vaccinating a chicken while at the same time immunising it against a protein. Antibodies can subsequently be isolated from the chicken's eggs in a process that generally does not cause the chicken any more distress than the current routine vaccination of production chickens.

Research projects 2014

As described in the Danish 3R-Center's 2014 annual report, the Center launched the following four research projects together with the then Minister for Food, Dan Jørgensen:

REFINING ANIMAL MODELS FOR PAIN RESEARCH: DEVELOPING METHODS TO ALLEVIATE PAIN IN RAT MODELS FOR PAIN RESEARCH

Klas Abelson, University of Copenhagen

Statement from Klas Abelson to the 3R-Center (11 December 2015): *"It is still a bit too soon to draw major conclusions as to whether it is possible to treat pain in the animal models, since the data processing has only just been initiated. The results of the project are expected to be published in the course of 2016."*

(Read more about the project at 3rcenter.dk)

STANDARDISING GUT MICROBIOTA IN MICE AS A TOOL FOR REDUCING THE NUMBER OF ANIMALS IN THE INDIVIDUAL EXPERIMENTS

Axel Kornerup Hansen, University of Copenhagen

Statement from Axel Kornerup Hansen to the 3R-Center (14 December 2015): *"The study has been completed and provides interesting data as it has been possible to create mice with a higher response, measured by the most important markers. A publication is expected in the spring of 2016."*

(Read more about the project at 3rcenter.dk)

PATHOLOGICAL AND IMMUNOLOGICAL CONSEQUENCES OF BLOOD SAMPLING IN MICE

Dorte Bratbo Sørensen, University of Copenhagen

Statement from Dorte Bratbo Sørensen (10 December 2015): *"All tissue analyses have now been completed and statistical data processing performed. We are writing up the results. The first draft of the article is expected to be ready by February/March 2016."*

(Read more about the project at 3rcenter.dk)

ARTIFICIAL BLOOD VESSELS – A MODEL FOR INVESTIGATING DIABETIC ATHEROSCLEROSIS

Mette Bjerre, Aarhus University

Statement from Mette Bjerre (5 November 2015): *"With the support from the Danish 3R-Center, we have established and optimised our human cell model, a type of artificial blood vessel that offers a unique opportunity for investigating the importance of blood sugar for the subsequent signalling between the cells. We also have interesting pilot data from the cell migration analysis that will help evaluate the functional importance of the changed signalling."*

Our next step is to examine the effect of various circulating diabetic blood markers on the cells, thus mimicking the internal bodily conditions. In 2016, we also expect to be able to start examining samples from patients with type 2 diabetes."

(Read more about the project at 3rcenter.dk)



2 WEBSITE 3RCENTER.DK

A new website to support the 3R-Center's communicative obligations was given priority in 2015.

Towards this end, the Danish 3R-Center launched a new website in August 2015 that enables it to heighten its profile even more, both within the laboratory-animal community and to stakeholders in the general public.

The 3R-Center's website will consequently focus on disseminating research and news of particular relevance to persons in the field of laboratory animal science. The 3R-Center aims to give everyone, both in Denmark and abroad, the opportunity to stay up-to-date on the most important 3R research and 3R events.

The 3R-Center is preparing informative material on laboratory animals that it will publish in stages as the material is completed. The material is mainly based on information from the Danish Animal Experiments Inspectorate which holds large volumes of statistics about the use of laboratory animals. The Danish 3R-Center is working closely with the Animal Experiments Inspectorate in its daily activities to ensure that the 3R-Center can vouch for the information published.

The material is particularly relevant for lower- and upper-secondary school students looking for information about laboratory animals and the presentation at the website will therefore be in the form of popular science. The students can thus find answers to questions such as: *How many laboratory animals do we use in Denmark? Which laboratory animals are being used? What is an animal experiment? What are laboratory animals used for?*

The teaching materials recently developed by the 3R-Center will also be available in this section of the website.

Teaching materials on laboratory animals and the 3Rs

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

The materials were developed with a view to influencing “tomorrow’s researchers” to ensure that their future research includes an awareness of the existence of the 3Rs which can have a positive effect on animal testing. Aiko elaborates on the purpose of the materials: *“The primary objective is to give students insight into the nature of the 3Rs – an outline of the historical background, ethics, legislation and regulation as well as research that exemplifies working with the 3Rs.”*

The teaching materials consist of an introductory quiz, a background article, a film, assignments, a concluding quiz and a teacher’s book. Aiko’s experience with the target group played a decisive role in designing the materials. Aiko’s thoughts on the target group: *“The learning must be as simple and accessible as possible, as all students must be able to both read and understand the contents. It cannot be expected that all students are able readers and, similarly, it cannot be expected that all students are motivated to learn about the subject at all.”*

The Danish 3R-Center has been in contact with upper-secondary school teachers who have already used the teaching materials. One teacher used the materials in a biotech class that worked on the subject of *Drug development*, and the students were asked to incorporate the 3R mindset in the hypothetical development of new drugs.

In early 2016, the materials will be supplemented with an additional three articles based on three of the projects launched by the Danish 3R-Center.

The articles concern the following projects:

- Developing an *in vitro* method to predict acute pulmonary toxicity from aerosol proofing products (Søren Thor Larsen, The National Research Centre for the Working Environment)
- Can chickens be immunised with an aerosol combined with vaccination? Investigating a non-invasive method for producing antibodies (Otto Kalliokoski, University of Copenhagen)
- Pathological and immunological consequences of blood sampling in mice (Dorte Bratbo Sørensen, University of Copenhagen)

Aiko Sho Nielsen commented upon on the three articles: *“The educational focus of the scientific articles is on the 3Rs, but also on the core subject-matter for upper-secondary schools which means that these articles will use more specialised language than the rest of the material.”*

To generate awareness of the learning materials, the Danish 3R-Center sent e-mails and letters to all upper-secondary educational institutions in Denmark. In this connection, any interested teachers have been invited to a presentation of the teaching materials (Spring 2016) to better prepare the teachers for the teaching situation.

Press coverage of the Danish 3R-Center

The distribution of research grants gave the Danish 3R-Center particular mention in printed and digital media (daily newspapers such as *Berlingske*, *Århus Stiftstidende*, *Fyns Amts Avis*, *Kristelig Dagblad*, *BT*, and *Ekstra Bladet*).

As the Danish 3R-Center is still in its start-up phase, it is important that the centre's work is also visible outside the laboratory animal environment, as increased knowledge of the existence of the 3R concept can ultimately enhance the implementation of the 3Rs. The 3R-Center is therefore making an effort to attract media attention. In 2016 this will specifically entail issuing press releases in cooperation with the Danish Veterinary and Food Administration's PR department in connection with the allocation of research funds, the annual symposium, the 3R award and other events that can showcase the 3R-Center's and the minister's efforts for laboratory animals.

Chickens to lay eggs for new experiments

Anne Louise Mikkelsen (landbrugnord.landbrugnet.dk),
10 February 2015

An experimental project sets out to examine whether chickens can replace rabbits and goats in the production of antibodies.

According to Dan Jørgensen (Social Democratic Party), the Danish Minister for Food, the number of laboratory animals must be reduced. For this purpose the minister will allocate grants to three projects conducting research into alternatives to animal testing.

"There are currently no viable alternatives to all the animal testing that is being carried out to develop essential human medicines. As a consequence, we must constantly look for new alternatives in order to reduce the use of live animals", says Dan Jørgensen in a statement on the Ministry of Food website.

One of the research projects will examine how chickens can be used instead of rabbits and goats in the production of antibodies for medicine. Using chickens eliminates the need to draw blood as antibodies are excreted in large quantities in the eggs.

Possible help against MRSA infections

The other project will attempt to develop a lung model that enables the testing of a vast range of chemicals that are currently being tested in animals, whereas the third project will develop artificial skin to be used to test new medicines against skin infection.

"The three projects are very interesting and will be used to conduct research into damage to human lungs and MRSA skin infections, for instance. I hope that the projects are successful", says Dan Jørgensen.

The Danish Ministry for Food has granted DKK 1.3 million for the three projects and the Danish 3R-Center is responsible for allocating the funds.



3 3R EVENTS

Each year, the Danish 3R-Center organises a symposium, and representatives of the 3R-Center also participate in relevant events in Denmark and internationally.

The symposium and some of these events are both described below, as this provides a good idea of the current 3R efforts in Denmark and the EU.

World Day for Laboratory Animals

24 APRIL 2015

DOSO and the Danish Animal Welfare Society organised the Danish participation in the World Day for Laboratory Animals for the fourth consecutive year.

This year's theme was *Refinement – the Danish effort to refine animal testing* – with a series of interesting presentations that gave a good impression of how refinement is exercised in Denmark and abroad. The theme is well aligned with the trend – that is also seen internationally – of increased focus on refinement, realising that not all laboratory animals can currently be replaced by alternatives, but without compromising on the task of achieving the ultimate goal of a laboratory-animal-free research world.

The Danish 3R-Center was represented on the programme by Christine Nellemann who gave a presentation on Priority Areas of the Danish 3R-Center and Strengthening of Refinement in 2015. In addition to describing the general work of the 3R-Center, her presentation focussed upon the three refinement projects supported by the 3R-Center in 2014 and 2015:

- *Can chickens be immunised with an aerosol combined with vaccination? Investigating a non-invasive method for producing antibodies* (Otto Kalliokoski)
- *Refining animal models for pain research: Developing methods to alleviate pain in rat models for pain research* (Klas Abelson)
- *Pathological and immunological consequences of blood sampling in mice* (Dorte Bratbo Sørensen).

The Danish 3R-Center's symposium

10. – 11. NOVEMBER 2015

The annual symposium is a significant event in efforts to promote the development of the 3Rs in Denmark, as it affords the laboratory animal community a chance to become acquainted with both national and international 3R research and take away inspiration and valuable lessons.

The Danish 3R-Center's 2015 Symposium was held in Copenhagen on 10-11 November. More than 140 participants benefited from a multi-faceted programme that also provided ample time for networking with peers and colleagues (see abstracts and presentations from the symposium at 3rcenter.dk).

The year's programme

10. NOVEMBER 2015

This year's host was Lisbeth E. Knudsen from the 3R-Center's Board. She opened the symposium with her thoughts on the trend in the use of laboratory animals in Europe and a glimpse of the future with respect to developing alternatives. She then moved on to introduce the symposium's first speaker, Christine Nellemann, chairman of the Board of the Danish 3R-Center.

“The symposium was excellent and featured very interesting talks”

“The presenter from the UK Centre for the 3Rs was very interesting”



THE DANISH 3R-CENTER: PROJECTS AND PLANS

Christine Nellemann, the Danish 3R-Center

Christine Nellemann gave an account of the factual background for the coming into existence of the centre and its financial situation, after which she presented the work of the Danish 3R-Center in 2015.

EXPERIENCES FROM THE UK 3RS CENTRE: CURRENT ACTIVITIES AND FUTURE PLANS

Ian Ragan, NC3Rs

The next speaker was Ian Ragan of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The British government set up the NC3Rs in 2014 with an annual budget of GBP 10 million.

The presentation focused on the following three themes in particular:

CRACK IT, Experimental Design Assistant (EDA) and the NC3Rs' recent focus on 3R implementation in companies. CRACK IT aims to accelerate the development, application and commercialisation of technologies with 3R potential by facilitating partnering between the industry, the research community and SMEs (<http://www.crackit.org.uk>).

CRACK IT is a competition of sorts about research funds where a company can approach the NC3Rs with a request to resolve a 3R-related issue. Individuals and companies that believe they can solve the issue at hand can then contact the NC3Rs who will award funding to the best solution for realising the project.

Experimental Design Assistant (EDA) is a free, web-based tool to support researchers' planning of animal testing and thereby ensure a good experiment with reliable and reproducible results. EDA was developed by the NC3Rs in a partnership between *in vivo* researchers, statisticians, academia and industry as well as software designers specialised in artificial intelligence (<https://eda.nc3rs.org.uk>).

The last topic of the presentation concerned the NC3Rs' recent focus on 3R implementation in companies. The NC3Rs will generally take on an advisory role and communicate its work to companies.

The NC3Rs will also offer access to the latest 3R knowledge for university researchers involved in *in vivo* research and will constantly stay abreast of research and technology with 3Rs potential and connect this with potential end users. It will also facilitate enhanced knowledge-sharing between companies.

SYSTEMATIC REVIEWS AND META-ANALYSES OF ANIMAL EXPERIMENTAL STUDIES – IMPROVING RESEARCH AND IMPLEMENTING THE 3 RS?

Birgitte Kousholt, Aarhus University

This presentation queried the lack of tradition among researchers with respect to carrying out Systematic Reviews (SR) and Meta-Analyses (MA), depriving them of the full overview of the knowledge that is already available and the research that is already being undertaken.

Birgitte Kousholt presented arguments in favour of the benefits provided by the studies – also in the field of 3R – where repeated experiments could possibly be avoided, thus reducing the number of laboratory animals (reduction).

Systematic review: A evidence-based literature search based on a single research question puts the same level of rigor to reviewing research evidence as should be used producing the research in the first place

Meta-analysis: Use of statistical methods to summarize the results of independent studies.

At Aarhus University's Department of Clinical Medicine, SR and MA are given high priority and are the most recent 3R initiatives of the department. This is also why this department held a symposium and workshop covering this topic on 17–18 November 2015 with support from the Danish 3R-Center.

Symposium – 10 November 2015

9.00-10.00	Registration and breakfast	
10.00-10.15	Welcome by moderator	Lisbeth E. Knudsen, The Danish 3R-Center
10.15-10.45	The Danish 3R-Center: Projects and plans	Christine Lydia Nellemann, The Danish 3R-Center
10.45-11.30	Experiences from the UK 3Rs Centre: Current activities and future plans	Ian Ragan, NC3R
11.30-12.00	Systematic reviews and meta-analyses of Animal Experimental Studies – improving research and implementing the 3Rs	Birgitte Kousholt, University of Aarhus
12.00-13.00	Lunch	
13.00-13.30	Execution of Novo Nordisk Animal Ethics Ambition, The establishment of a 3R department	Stine Øvlisen, Novo Nordisk
13.30-13.50	Completely Replacing All Invasive Elements in Polyclonal Antibody Production – Possibilities and Challenges with Using Chickens	Otto Kalliokoski, University of Copenhagen
13.50-14.10	Development of an <i>in vitro</i> human skin model for evaluation of topical antimicrobial compounds	Mette Elena Skindersø, University of Denmark
14.10-14.30	Development of an <i>in vitro</i> model to predict acute lung toxicity of waterproofing spray products	Søren Thor Larsen, National Research Centre for the Working Environment
14.30-15.00	Break – networking	
15.00-15.30	Teaching the Three R's – conveying the message to Danish students	Aiko Sho Nielsen, Freelance journalist
15.30-16.00	The 3Rs: on animal scientists perceptions, awareness and practices	Jesper Lassen, University of Copenhagen
16.00-16.30	Looking at data – 10 years of data from the Danish Experiments Inspectorate	Louise Bjørn Brønden, The Danish 3R-Center
16.30	Networking	

Symposium – 11. november 2015

9.00-9.30	Moving forward with the Three Rs in the EU	Susanna Louhimies, The European Commission
9.30-10.00	Filling information gaps with QSAR predictions for 600,000 chemical substances for Reduction, Replacement, Prioritization and Substitution	Eva Bay Wedebye, Danish Technical University
10.00-10.30	Toxicogenomics approaches towards predicting chemical carcinogenicity <i>in vitro</i>	Jos Kleinjans, Maastricht University
10.30-11.00	Break – networking	
11.00-11.30	Stem cell models – hypes and hopes in their potential as replacement for animal testing	Christian Clausen, Danish Stem Cell Society (DASCS)
11.30-12.00	Human pain experiments as an alternative to animal models	Asbjørn Mohr Drewes, Aalborg University Hospital
12.00-12.30	Danish 3R-award 2015	
12.30-13.30	Lunch	
13.30-14.15	Engineering of human 3D vascularized tissues including disease models	Angela Rossi, Fraunhofer Institute
14.15-15.00	The history of laboratory animals and the 3Rs	Aage Kristian Olsen Alstrup, University of Aarhus
15.00	Thank you and goodbye by moderator	Lisbeth E. Knudsen, The Danish 3R-Center

EXECUTION OF NOVO NORDISK ANIMAL ETHICS AMBITION, THE ESTABLISHMENT OF A 3R DEPARTMENT

Stine Øvlisen, Novo Nordisk

Stine Øvlisen gave an account of the considerations underlying the establishment of a 3R department at Novo Nordisk and told about the future work of the department.

By way of example, she explained that it is not a simple task to be a growing, global company which is under a 3R obligation – while constantly wanting to improve the 3R efforts – and that Novo Nordisk has consequently established this department to promote innovation in the 3R field. The department will contribute to an even more efficient integration of 3R considerations in decision-making processes, and the department will also assist the company's animal keepers and researchers with conceptual 3R development and implementation.

The department will also engage in dialogue with the company's main stakeholders and set up partnerships and collaboration.

COMPLETELY REPLACING ALL INVASIVE ELEMENTS IN POLYCLONAL ANTIBODY PRODUCTION – POSSIBILITIES AND CHALLENGES WITH USING CHICKENS

Otto Kalliokoski, University of Copenhagen

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

DEVELOPMENT OF AN IN VITRO HUMAN SKIN MODEL FOR EVALUATION OF TOPICAL ANTIMICROBIAL COMPOUNDS

Mette Elena Skindersø, Technical University of Denmark

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

DEVELOPMENT OF AN IN VITRO MODEL TO PREDICT ACUTE LUNG TOXICITY OF WATERPROOFING SPRAY PRODUCTS

Søren Thor Larsen, The National Research Centre for the Working Environment

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

TEACHING THE THREE R'S – CONVEYING THE MESSAGE TO DANISH STUDENTS

Aiko Sho Nielsen, Freelance journalist

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

THE 3RS – ON ANIMAL SCIENTISTS PERCEPTIONS, AWARENESS AND PRACTICES (LISBETH E. KNUDSEN HELD THE LECTURE ON BEHALF OF JESPER LASSEN)

Jesper Lassen, University of Copenhagen

This talk presented the initial results of the study funded by the Danish 3R-Center about the 3R knowledge and experience of laboratory-animal users, and their idea of the concept is also included in the study.

Most respondents in the study do not believe that there is a conflict between research quality and implementation of the 3Rs, and many do not experience any obstacles in relation to implementing the 3Rs in their work.

Offhand, the initial data analyses appear to indicate that the most important obstacle in relation to reducing the number of animals in research is the lack of cooperation between scientists, researcher groups and companies. The report is expected to be published in 2016.

The year's programme

11. NOVEMBER 2015

LOOKING AT DATA – 10 YEARS OF DATA FROM THE DANISH EXPERIMENTS INSPECTORATE

Louise Bjørn Brønden, the Danish 3R-Center

Approximately 240,000 laboratory animals were used in Denmark in 2013.

The number has declined in recent years, and the Danish 3R-Center launched a survey in 2015 to shed light on the reasons for this decline. In the autumn of 2015, a statistician reviewed the data for the past ten years, which will now be subject to detailed analysis.

Louise Bjørn Brønden told the audience how the analysis will be performed.

The report is expected to be published in 2016.

MOVING FORWARD WITH THE THREE RS IN THE EU

Susanna Louhimies, European Commission

Susanna Louhimies based her presentation on EU Directive 2010/63/EU on the protection of animals used for scientific purposes – a directive that today is an integral part of the legislation on laboratory animals in the individual member states. She informed the symposium that all EU member states have a duty to launch various initiatives to promote 3R.

Susanna believes that the Danish 3R-Center is an excellent example of such an initiative that the Commission hopes will spread to other countries. Susanna told how EURL-ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) had updated and reissued their search guide *Good search practice on animal alternatives*, which will help researchers, ethical committees and similar who are not familiar with the use of the many existing databases to search for alternatives to animal testing and thus prevent animal testing being performed if there is an alternative.

The last part of the presentation dealt with the concept of *Culture of Care*, which covers not only compliance with legislation, but also building a culture in the company/institution with a constant focus on improvements – a culture in which everyone assumes responsibility for disseminating and implementing the 3Rs.

“Very nice programme with interesting subjects. The presentations from the NC3Rs and on the EU Directive were very interesting”

FILLING INFORMATION GAPS WITH QSAR PREDICTIONS FOR 600,000 CHEMICAL SUBSTANCES FOR REDUCTION, REPLACEMENT, PRIORITIZATION AND SUBSTITUTION

Eva Bay Wedebye, Technical University of Denmark

Definition of QSAR (Quantitative Structure-Activity Relationship): A QSAR is a mathematical model (often a statistical correlation) that relates one or more parameters derived from a chemical structure to a property or activity.

DTU's QSAR database contains estimates of the hazardous properties of no less than 600,000 substances of relevance to human health and the environment – be they substances which cause acute toxicity in rats, mice, fish, daphnia and algae, a vast array of physical-chemical and environmental properties, skin irritation, hypersensitivity, mutagenicity, cancer and reprotoxicity.

In short, QSAR is about predicting the health and environmental risks of chemicals based on large amounts of empirical data. Eva Bay Wedebye described the advantages provided by QSAR in the field of laboratory animals, as QSAR can replace the use of animals in some cases (replacement) and reduce the number of animals for an experiment in others (reduction).

In addition to a complete replacement of some experiments, QSAR can be used to support existing assessments and thus avoid additional testing.

In cases where animal testing is necessary, predictions of the mechanistic properties can contribute to optimising an experimental design to increase the body of knowledge that can be derived from the experiment without using additional animals.

TOXICOGENOMICS APPROACHES TOWARDS PREDICTING CHEMICAL CARCINOGENICITY IN VITRO

Jos Kleinjans, Maastricht University

The Department of Toxicogenomics is involved with predicting carcinogenicity *in vitro* using toxicogenomic approaches, which means examining gene and protein activity in response to toxic substances in the cells and tissue of an organism. The aim is to obtain an understanding of the potential carcinogenicity of a substance. *Toxicogenomics* thus combines toxicology and genome research.

The department endeavours to explore, develop and utilise the full potential of cell technologies and genomics platforms to promote mechanism-based *in vitro* analyses using human cells for predictive toxicology and to develop new biomarkers for toxic impact and effect to be used to replace existing animal models.

Finally, work is being done to develop a brand-new computer-based platform building on 3D organ models with human cells and data from existing cell technologies and databases, as well as knowledge from tissue analyses of patients treated with different medicines.

STEM CELL MODELS – HYPES AND HOPES IN THEIR POTENTIAL AS REPLACEMENT FOR ANIMAL TESTING

Christian Clausen, Bioneer A/S

Christian Clausen told the audience how stem-cell research has seen considerable progress over the past ten years. Combined with tremendous developments in, among other things, tissue engineering, it is tempting to conclude that stem-cell based models will be the path to animal-free research, according to the presenter.

Christian Clausen thus related how human stem cells harbour great potential, since it is possible to create 3D models in which the function of the entire organ can be replicated, and it is also possible to create disease-specific models that reflect the sick cell's reaction pattern.

HUMAN PAIN EXPERIMENTS AS AN ALTERNATIVE TO ANIMAL MODELS

Asbjørn Mohr Drewes, Aalborg University Hospital

Asbjørn Mohr Drewes's lecture dealt with the benefits of human models over animal models for pain experiments and how human models are consequently believed to be more suitable for examining the effect of analgesics.

Over the years, a vast number of animal models have been developed for exploring the pain system and examining the efficacy of analgesics, but pre-clinical animal models cannot correctly predict clinical efficacy in humans, and only a few models have been able to replicate the effect of analgesics in humans.

The human pain experiments take more account of the complexity associated with a clinical situation as, by contrast to animal models that are most often based on motor reflexes and behavioural reactions, they consider the factors that accompany the clinical situation in the form of, among other things, fear, nervousness, cognitive and autonomous reactions that affect the overall sensory experience.

Presentation of the Danish 3R-Center's 2015 3R award

The next item on the agenda for the day was the awards ceremony. The Danish 3R-Center's 3R award was presented to Hanne Gamst-Andersen of Novo Nordisk for her admirable and longstanding efforts in the field of laboratory animal science. Since the mid-1990s, Hanne Gamst-Andersen has been known as an ambassador for laboratory animals in the Novo Nordisk pharmaceutical company. During her time with Novo Nordisk and until her recent retirement, she has contributed strongly to the company's policy on the use of laboratory animals.

"Through her long-standing activities as a laboratory animal veterinarian with Novo Nordisk A/S, Hanne Gamst-Andersen has contributed significantly to improving conditions for laboratory animals. With her dedication and zeal, she has characterised the development of the 3Rs, not just at Novo Nordisk, but also externally," wrote Stine Øvlisen (Director, 3R Management & Strategy at Novo Nordisk) in her nomination of Hanne Gamst-Andersen.

Through her position at Novo Nordisk, Hanne Gamst-Andersen has been actively committed to developing the legislation in the area and has been acknowledged as a major capacity in the field by the rest of the pharmaceutical industry who have often sought her advice on laboratory animal welfare.

"Through much of her working life, Hanne Gamst-Andersen has practically personified what the 3R-Center stands for – which is to reduce the use of laboratory animals, find alternatives to animal testing and provide the best conditions for the animals that are still indispensable to the experiments. It therefore gives us great pleasure to present you with the 3R award, Hanne," emphasised Christine Nellemann when she presented the 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 that works to promote the 3Rs in Denmark.

The award is presented at the annual symposium. In the time leading up to the symposium, nominations can be sent to the Danish 3R-Center, following which the board decides who should receive the award, which comes with a diploma and DKK 10,000.

ENGINEERING OF HUMAN 3D VASCULARIZED TISSUES INCLUDING DISEASE MODELS

Angela Rossi, Angela Rossi, University of Würzburg

With her presentation, Angela Rossi gave a sneak preview of a world of three-dimensional biological models. The Fraunhofer Institute at the University of Würzburg has developed a 3D model from a piece of the small intestine of a pig. In order to get a tissue skeleton on which to build, the scientists first cleared away the existing cells from the small intestine tissue and then constructed a new intestine wall on the remaining tissue skeleton (BioVasc) using human skin cells cultivated in a cell culture. The model blazes new trails as it has been possible to recreate the branching of blood vessels in the tissue to a large extent. The researchers therefore have a biological model on their hands which closely resembles real human skin. The skin model is already in use at the institute. The applications include a study of wound healing and the carrying out of infection studies with tropical skin parasites. The Fraunhofer Institute also works on 3D models for the trachea, lungs and heart – all based on the BioVasc principle.

THE HISTORY OF LABORATORY ANIMALS AND THE 3RS

Aage Kristian Olsen Alstrup, Aarhus University

The last presentation of the day was Aage Kristian Olsen Alstrup's historical review of the use of laboratory animals with focus on the 3Rs. The symposium participants thus learned about how animal testing was performed in times past and how the suffering of the animals was defended. Of course, the presentation included the two initiators of the 3R concept, William M.S. Russell and Rex L. Burch, who introduced the concept in their *The Principles of Humane Experimental Technique* (1959). Today, the 3R concept has been incorporated into laboratory animal legislation in many countries, including the EU Directive and Danish law.

Aage Kristian Olsen Alstrup, speaker at the Danish 3R-Center symposium, authored an article on the symposium and the 3Rs for the science portal videnskab.dk, which the Danish 3R-Center warmly recommends (<http://videnskab.dk/blog/fokus-pa-de-3-r-er-replacement-reduction-og-refinement>).

“Great program and good balance between technical and more general presentations.”

“I would like to see a more practical approach next year – possibly with sharing of lessons learnt at a purely practical level. I believe we could use that in Denmark”

Satisfaction survey on the Symposium

After its evaluation of the symposium, the Danish 3R-Center noted that the participants considered the symposium to be a great success. More than half of the symposium participants took the time to evaluate the event, being asked to illustrate their satisfaction (or the opposite) with a rating from 1 (dissatisfied) to 5 (highly satisfied), which resulted in an average rating of no less than 4.3.

A few of the symposium's participants expressed their desire for a programme that included more practical work. The Danish 3R-Center is therefore contemplating whether it is possible to open parts of the meeting *Networking and dialogue on the function of animal welfare organs* to the public. The meeting is an annual event like the symposium but with far greater focus on practical sessions and experience-sharing, and making it open to the public would accommodate the wishes of animal carers and related stakeholders who can benefit greatly from this event.

Symposium and workshop about Systematic Reviews and Meta-analyses

17.-18. NOVEMBER 2015

The purpose of the symposium and workshop was to give the participants greater insight into Systematic Reviews (SR) and Meta-Analyses (MA) of animal studies as they are central for identifying the existing body of available knowledge and current research in a specific area.

Presenters from SYRCLE (Systematic Review Centre for Laboratory animal Experimentation) and CAMARADES (The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) guided the participants through both the symposium and the workshop. For many years, they have specialised in performing this particular type of searches and analyses.

At the symposium, talks were given about Systematic Reviews in the past, present and future. In addition, principles, methods and standards for SR were reviewed and examples were given of the value that SR has added to animal studies. The speakers illustrated how SR can facilitate the performance of 3R and thus contribute to improving the quality of animal testing.

The workshop reviewed tools for performing exhaustive literature searches and included practical, hands-on sessions with literature searches in databases.

All in all, the days offered a good learning experience focusing on the importance of good preparation and analysis prior to any animal testing with a view to optimising the tests, thereby ensuring that animals are only used and experiments are only made if they are absolutely necessary and if they have not already been performed.

SYRCLE is an institution in the Netherlands, at the Radboud University Nijmegen, which offers online introductions, hands-on training and coaching of scientists performing their own Systematic Reviews. They develop methodological tools and guidelines and conduct their own, systematic reviews of animal testing.

CAMARADES, a centre at the University of Edinburgh, offers networking contributions to groups involved in Systematic Reviews and meta-analyses of data from laboratory animal studies.

3R activities at Aarhus University

DECEMBER 2015

The Danish 3R-Center supported the symposium/workshop on Systematic Reviews and Meta-Analyses held by Aarhus University, and the 3R-Center has therefore asked N. Frederik Dagnæs-Hansen, Mette Herskind, Birgitte Kousholt, Aage Kristian Olsen Alstrup and Tobias Wang to give a description of elements of the 3R efforts undertaken at Aarhus University.

FACULTY OF HEALTH, DEPARTMENT OF CLINICAL MEDICINE

The Department of Clinical Medicine has launched 3R initiatives for research and teaching, legislation, implementation of management tools for animal-experiment studies as well as anaesthesia and analgesics. The 3R efforts take place concurrently in the daily work in the animal facilities.

In the fields of research and teaching, the department works with international partners on *systematic reviews and meta-analyses of pre-clinical animal studies*.

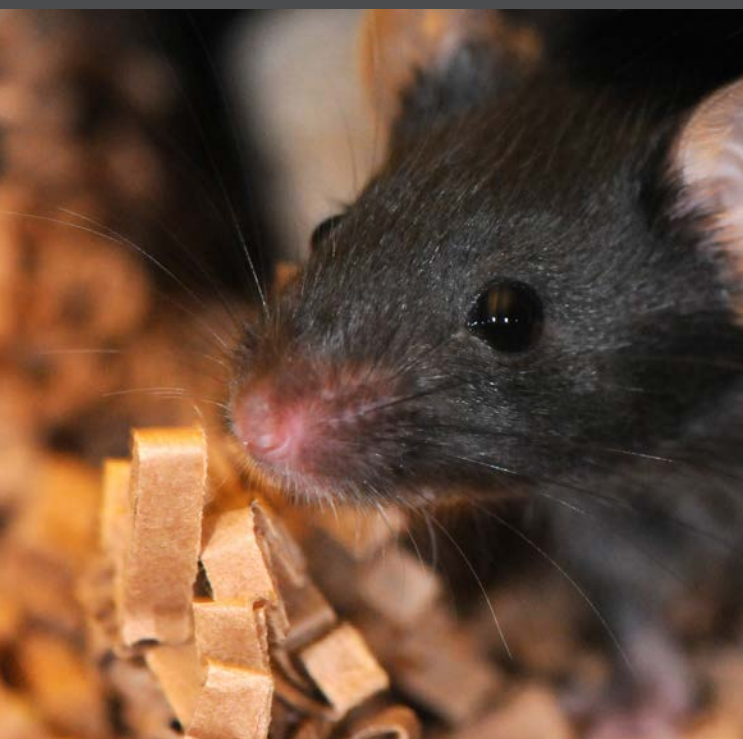
Systematic reviews (SR) and meta-analyses (MA) are the core of evidence-based health science research. It is a

thorough and systematic analysis of a specific research question where the goal is to find answers from already existing data. There is little tradition for SR and MA of pre-clinical animal studies at research institutions in Denmark, but this is absolutely necessary. One consequence of the effort is that the 3Rs are implemented already from the beginning of one's research project.

Implementing the SR and MA method is expected to lower the number of animal-experiment projects (reduction) and change the nature of such projects and further refine the animal testing that needs to be carried out.

In addition, the initiative will produce an even more solid research output that is rooted in already existing knowledge. Since 2014, SR has been taught at the animal experiment courses. The first symposium and workshop on SR and MA of pre-clinical animal studies in Denmark were held in November 2015 at the Department of Clinical Medicine. The event was well-attended by the department's employees and students, other AU divisions, but also attracted visitors from the rest of Denmark and Norway.

The department has also invested in a brand-new management tool that provides an overview of all animal testing carried out at the department. It covers relevant processes for compliance with permits, operations and reporting, but also animal welfare. It provides a single point of entry to the projects for all involved groups of professionals – from researchers, veterinarians, students and animal keepers to the management. The implementation of the system began in 2015 and will continue in 2016.



The veterinary unit also works with refinement in the form of improvements of current anaesthetic and analgesics protocols. The work is carried out in close contact with the individual research groups and specialists in veterinary anaesthetics and analgesics at national and international levels. This effort will be permanent and is expected to be enhanced over the coming five years.

In an effort to improve general animal welfare, work is also being done to optimise the general state of health of the laboratory animals. A good state of health helps minimise the number of animals used in experiments. Several units in the animal facilities pursue specific measures to enrich the daily welfare for the animals and their possibilities of exhibiting normal behaviour. This involves approaches such as using other types of bedding, shelter, nesting materials and feeding balls.

A course was held in 2015 on anaesthesia and analgesia of pigs used in experiments. The course is expected to be offered again.

DEPARTMENT OF BIOMEDICINE

At the Department of Biomedicine, high priority is given to the 3Rs. In particular, resources are expended on training future researchers with a view to implementing the 3Rs in future research activities. The department has also worked on improving non-invasive examination methods that use scanning techniques and making them more efficient so that the same animal can be used in more studies, which reduces the number of laboratory animals used. By exploring methods for applying and lifting anaesthesia, the department is also seeking to improve the potential for reducing the distress induced by anaesthesia and also for restoring normal physiological conditions in the animals after anaesthesia, thus ensuring more correct and reproducible data.

Another initiative that also helps implement the 3Rs at the Department of Biomedicine is a modernisation and revision of the methods for controlling the health of the laboratory animals. As a result of the new methods, fewer animals are used for checking the animals' state of health and thus ensure that animals of the highest quality are always used in the animal testing considered necessary to improve the treatment and diagnosis of diseases in people.

SCIENCE & TECHNOLOGY, DEPARTMENT OF ANIMAL SCIENCE

At Aarhus University, Science & Technology, animal experiments is an integral part of the research and education carried out at the Department of Animal Science as well as the Department of Bioscience. At the Department of Animal Science, animal welfare is a central research issue that also comprises laboratory animal welfare – focusing on species such as pigs and cows that have traditionally been included in research that focuses on food production. However, the use of pigs in particular as a laboratory animal and animal model has sharply increased in recent years. The department's researchers have long-standing experience of pain studies in these species and participate in joint efforts with relevant researchers in Denmark and abroad to establish knowledge in this field – with particular focus on refinement of the conditions for the laboratory animal.

Across the department, a number of experiments that require permits are also being conducted, focusing on food production and on the pig as a model for human nutrition. The 3Rs are included in the department's courses at Master and PhD level and have been incorporated and prioritised in the department's animal welfare policy. In recent years, the department's researchers have participated as instructors on an expanded laboratory animal course (jointly held with the Department of Bioscience) that includes the use of domestic animals and/or exotic animals.

DEPARTMENT OF BIOSCIENCE

Since 2012, the Department of Bioscience has regularly offered a special laboratory animal course (FELASA category B) that focuses on exotic laboratory animals with emphasis on the special considerations required to secure the 3Rs during experiments with ectothermic vertebrates. This course thus helps improve animal welfare (refinement) in experiments involving fish, amphibians and reptiles. The course has also been held twice in Brazil on request. In 2015, an advanced laboratory-animal course (FELASA category C) was also held at the Department of Bioscience, focusing on exotic laboratory animals and field testing. Furthermore, a broad partnership including partners such as the Copenhagen Zoo and the Department of Clinical Medicine with associated PhD and Master students conducts a series of investigations into improved pain relief and anaesthetic methods in amphibians and reptiles.

Networking and dialogue about the function of animal welfare bodies

15. DECEMBER 2015

Together with a steering group, the Committee for Laboratory Animals and Alternatives organised the meeting with representatives of most of the Denmark's animal welfare bodies.

The programme opened with a presentation by the Committee for Laboratory Animals and Alternatives held by Christine Nellemann, after which three animal-welfare bodies from three different laboratory-animal facilities were presented (Roche, Statens Serum Institut and Novo Nordisk).

The Danish 3R-Center and the Committee for Laboratory Animals and Alternatives are of the opinion that the animal-welfare bodies probably represent a good forum from which a *culture of care* can originate, as explained by Susanne Louhimies of the European Commission at the Danish 3R-Center's symposium. Indeed, the relevance of the animal-welfare bodies was made evident by the three presenters.

This was followed by group work on the purpose of the animal-welfare bodies and how knowledge can be best shared among the animal-welfare bodies. It was also discussed how the Committee for Laboratory Animals and Alternatives can best support the animal-welfare bodies in their work.

An important part of the programme was to let representatives of the animal-welfare bodies share and exchange good ideas that somehow have had a positive impact on the laboratory-animal area in their respective experiment sites. Representatives of 31 of Denmark's 42 animal-welfare bodies participated in the event, and many chose to present refinement issues in particular, for instance in the form of animal facility enrichment.

In this connection, the Committee for Laboratory Animals and Alternatives has set up a site at the website of the Danish Veterinary and Food Administration to facilitate the exchange of good ideas/3R initiatives for inspiration (http://www.foedevarestyrelsen.dk/fvst_ansvar_opgaver/Sider/Udvalget-for-Forsogsdyr-og-Alternativer).

The Committee for Laboratory Animals and Alternatives

The Committee has a chairman and six other members with a high level of professional knowledge and experience in at least one of the following areas: Replacement, Reduction and Refinement in connection with animal testing. The Committee chairman is the chairman of the Board of the 3R-Center, and the six other members of the Committee are the other six members of the Board of the 3R-Center.

The Committee will promote the use of 3R principles in relation to animal testing and must ensure that the animal-welfare bodies are given advice on issues concerning the acquisition, breeding, housing, care and use of laboratory animals, and the Committee must also facilitate the exchange of best practices.





4 INTERNATIONAL COOPERATION

Ever since the Danish 3R-Center was established, it has been essential to the Ministry of Environment and Food of Denmark, stakeholders and the board that the Centre focuses on international collaboration.

The use of laboratory animals is not restricted by national borders but takes place as international cooperation, e.g. among multinational pharmaceutical and chemicals companies, research partnerships between universities and through the exchange of researchers from many countries.

At the same time, the Danish 3R-Center, despite its many activities, is a comparatively small compared with sister organisations in the UK (NC3Rs) and Germany (ZEBET), for instance.

The Danish 3R-Center can achieve faster – and perhaps avoid beginner's errors – if it takes inspiration from colleagues from all over the world, and setting up collaboration with foreign centres can strengthen the Centre's work. Accordingly, the Danish 3R-Center was already working to forge relations with similar centres all over the world in 2014.



Finnish Centre for Alternative Methods (FICAM)

In January 2015, representatives of the Danish 3R-Center visited FICAM in Tampere, Finland. FICAM develops and validates tissue and organ models based on human cells, informs about alternative models, trains experts and serves as Finland's reference laboratory for validating alternatives to animal testing. The centre is located at Tampere University under the management of Tuula Heinonen who has longstanding experience of using alternatives for animal testing in his capacity as toxicologist.

With its focus on replacement, FICAM is an obvious partner for the Danish 3R-Center. For instance, it will be possible to cooperate on further developing and validating models originally developed by Danish researchers, and updated knowledge of developments in the field can be communicated to Danish researchers.

European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM)

In April 2015, the Danish 3R-Center participated in the first joint meeting for 3R centres in Europe. The meeting was called by EURL-ECVAM which is the EU joint centre for validating alternative methods and the European Union Reference Laboratory.

In addition to employees from EURL-ECVAM, the meeting was attended by representatives from centres in Sweden, Norway, Finland, Denmark, Germany, the Netherlands, Italy, the UK and Romania – the latter had just established a 3R centre. Even though it is evident that a number of EU member states are still missing, it still goes to show that the idea of building dedicated 3R centres is gaining ground.

The meeting was held in an atmosphere of openness and cooperation and resulted in the following conclusions:

Existing 3R centres vary greatly in terms of structure, affiliation with official functions, focus areas and financing. Some centres should be considered NGOs that attempt to strengthen 3R developments for private funding. Others are more scientifically based and often located at universities receiving various degrees of government support, whereas others, such as the Danish centre, are mainly public-sector organisations.

Despite the differences, it was also clear that there was an obvious potential for collaboration and thus for strengthening the effort at international level.

Six topics of interest to all participants/centres were identified. They will form the basis for further discussions on any future joint efforts. The Danish 3R-Center's board will be represented in all six discussion groups. The six topics are:

- Identification of priorities for reducing the number of laboratory animals in biomedical research – including addressing concerns with respect to the increasing use of transgenic animals.
- Communication and dissemination
- Promoting the use of alternative methods/models as a biotechnical resource – including characterisation and standardisation
- Education and training
- Validation in relation to legislative recognition
- Research support awarded by 3R centres

The refinement trend that was already evident at the World Congress on Alternatives and Animal Use in the Life Sciences in Prague in 2014 continued at this meeting. While the main focus was previously on developing alternatives to laboratory animals, recognition has emerged that laboratory animals are still necessary in many cases and cannot yet be replaced entirely. Therefore, their conditions must be as good as at all possible (refinement). As is the case for the Danish 3R-Center, some centres already focus intensely on refinement, while others are in the process of strengthening the efforts in this area in their organisation.

European Society for Alternatives to Animal Testing (EUSAAT)

In September 2015, representatives of the Danish 3R-Center participated in the 19th European Congress on Alternatives to Animal Testing which is held in Linz, Austria every year. From previously being a local German-language event, the congress language is now English and it has grown to no less than three programme tracks with participants from more than 50 countries.

In addition to a great number of talks on the amazing developments currently taking place in the development of alternatives – including 3D cell models, human-on-a-chip, QSAR and stem cells – the trend of higher focus on refinement was made clear once more.

At the same time, it was evident that international cooperation on implementing common rules and procedures will benefit all.

The Danish 3R-Center presented a poster showing its structure and projects, but equally importantly, the Linz meeting provided yet another opportunity to meet international colleagues and share experiences, ideas and plans.

In Sweden, as in countries such as Canada and, to some extent, in the UK, there is extensive cooperation on animal welfare across the board, be it production animals, companion animals or laboratory animals. Refinement is a question of animal welfare, and the animals' physical and behavioural needs are the same, regardless of whether they are used for experiments, bred for food or kept as pets.

Nordic platform for Communication on Animal Welfare (NordCAW)

In November 2015, the Danish 3R-Center and the Danish Veterinary and Food Administration's Animal Welfare Knowledge Centre (VID) co-organised a pan-Nordic conference on the communication of animal welfare issues. The main organiser of the conference, which received support from the Nordic Council, was the Swedish Centre for Animal Welfare (SCAW) in Uppsala, Sweden, which has formed NordCAW together with sister organisations from Finland, Norway, Estonia, Lithuania, the Faroe Islands and Denmark.

In Sweden, as in countries such as Canada and, to some extent, in the UK, there is extensive cooperation on animal welfare across the board, be it production animals, companion animals or laboratory animals. Refinement is a question of animal welfare, and the animals' physical and behavioural needs are the same, regardless of whether they are used for experiments, bred for food or kept as pets. Pain and suffering can be assessed and relieved in the same way, notwithstanding whether the suffering is deliberately inflicted in an experiment or occurs as a result of improper care and supervision in a production process.

The actual conference consisted of a morning meeting at which a variety of initiatives for communicating knowledge on animal welfare were presented – for instance an excellent animated film from the Faroe Islands that presented a new and gentler way to hoist up sheep on steep cliffs.

In the afternoon, there were three parallel sessions on how to communicate issues concerning pig herds, animal slaughtering and the use of laboratory animals. The Danish 3R-Center organised the latter session at which a representative from the Animal Experiments Directorate told about the longstanding tradition of publishing animal-testing permits online. Stine Øvlisen of Novo Nordisk told about the company's policy on openness concerning the use of laboratory animals, and Emma Sanchez of the *European Animal Research Association* argued in favour of why such openness is necessary everywhere in order for the general public to continue accepting the use of laboratory animals.

All in all, it was an interesting day with inspiration freely flowing among countries and a variety of animal users.

EU

Ever since the new EU Directive on laboratory animals came into effect, the European Commission has worked intensely, not only to ensure the implementation of the new rules but also to launch 3R initiatives throughout Europe. This both concerns the establishment and operation of animal welfare bodies to be present in all locations at which laboratory animals are used in the EU, as well as other 3R initiatives that all member states are obliged to implement under the directive.

At its two annual meetings, the European Commission and the member states (with Denmark being represented by the Danish Veterinary and Food Administration) discuss these initiatives, and working groups are regularly set up to address different issues. The members include professional representatives from both the Danish Animal Experiments Directorate and the Danish 3R-Center.

The Commission held its first meeting for the National Committees in 2015.

The many initiatives can be followed on the European Commission website – including the projects carried out by individual member states.



5 APPENDIX

The Danish 3R-Center

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

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

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

This was not satisfactory for the groups of stakeholders, which was expressed in a stakeholder analysis carried out in 2011/2012 among all interested parties in the field of laboratory animals. Almost unanimously, the feedback was that Denmark should either set up a 3R-Center inspired by the British NC3Rs (National Centre for the Replacement, Refinement & Reduction of Animals in Research) and the German ZEBET (Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch am BfR) or allocate sufficient funding to DACOPA.

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

The Board of the Danish 3R-Center

CHRISTINE NELLEMANN (CHAIRMAN)

Christine Nellemann has a degree in human biology and a PhD from the Panum Institute. She works in the Division of Toxicology and Risk Assessment at the National Food Institute, Technical University of Denmark (DTU Food), where she is the Director of Institute.

Christine's 3R vision is that by taking an integrated approach to the use of cell models, computer models and animal models, it is possible to make great strides in predicting and recreating effects in humans. Christine can contribute the research and knowledge amassed at DTU Food within the use of QSAR (Quantitative Structure–Activity Relationship models), a battery of cell models and advanced animal models for predicting effects in humans.

Christine has the following goal for the 3R-Center's work: *"We must initiate new research in this area and render visible the efforts being made to replace animal testing, reduce the number of animal tests and improve conditions for laboratory animals."*

PETER BOLLEN

Peter Bollen is an Associate Professor and Head of Department at the University of Southern Denmark. Peter has worked with laboratory animals since 1992. His interests include anaesthesia improvements and optimising both the housing of laboratory animals and their nutrition. The latter was also the subject of his PhD studies.

It is Peter's vision that he will find new ways, together with the rest of the board, for increasing the knowledge of 3R principles in the research community as well as in society. Peter will also use his international network to forge contacts with a view to knowledge sharing.

Peter also has a goal of establishing a mindset among researchers where animal testing is not automatically made integral to a planned experiment. If animal testing cannot be avoided, the least strenuous methods should be applied and no more animals than strictly necessary should be used. This goal is best achieved through knowledge sharing – knowledge that can be obtained from projects such as those financially supported by the Danish 3R-Center.

AXEL KORNERUP HANSEN

Axel Kornerup Hansen is a veterinarian with a doctorate in veterinary science and a Professor of laboratory animal science and welfare at the University of Copenhagen. He conducts research in the areas of reduction and refinement – primarily in rodents.

Axel's vision for the 3R field is to ensure real improvements in the welfare of animals used for research and development and to optimise the scientific results achieved from the individual animal, thus reducing the number of animals needed in each experiment.

Axel interacts well with the research community and can therefore help initiate 3R activities at research laboratories. He can also contribute to convincing researchers that using fewer animals and optimising research methods are in their own best interests.

Axel believes that the purpose of the 3R-Center's work is to launch more 3R projects, for several reasons. For instance, refinement projects will enhance animal welfare which makes being a laboratory animal a bit more fun and also make scientists happier as they will subject their animals to less distress. Reduction projects that increase the efficacy of animal testing and thus also increase the utilisation rate of the laboratory animals and replacement projects that can result in animal-free testing are also positive aspects for the researchers.

LISBETH E. KNUDSEN

Lisbeth E. Knudsen has an MSc in toxicological biochemistry and a PhD in biomedicine and is a Professor in toxicology at the University of Copenhagen. Lisbeth's research includes the use of *in vitro* cell cultures and human tissue to document toxicological mechanisms and effects in humans subject to anthropogenic exposures. This can save animal testing. The data are used to prevent harm to human health and are part of the risk assessment made by authorities.

Lisbeth can contribute her highly comprehensive professional/scientific experience in 3R, nationally as well as internationally. She is the Vice-President of *ecopa* (European Consensus-Platform for Alternatives – www.ecopa.eu) and has been a member of ESAC (European Union Ref-

erence Laboratory for Alternatives to Animal Testing – EC-VAM Scientific Advisory Committee).

Lisbeth was also affiliated with DACOPA (Danish Consensus Platform for Alternatives to Animal Experiments), the predecessor of the Danish 3R-Center.

Lisbeth's idea of the goal for the 3R-Center's work: *"I have visions of drawing attention to animal-free examination methods, communicating 3R in Denmark to a broader audience and initiating research projects within animal-free studies, including developing a researcher training programme associated with Denmark's graduate research schools."*

JAN LUND OTTESEN

Jan Lund Ottesen is a qualified veterinarian, holds a PhD degree and has been involved with laboratory animals at Novo Nordisk for more than 25 years. He is also a European Veterinary Specialist in Laboratory Animal Medicine and a *de facto* diplomat of ECLAM (European College of Laboratory Animal Medicine).

Jan's vision for the 3R-Center involves that everyone adheres to the 3R principle so that only the number of animals necessary for answering the stated hypothesis are used; that the animals used have as good a life as they can get while in our care and that all of us do our utmost to use research tools that do not involve the use of animals. Jan is experienced in setting and achieving annual goals and developing 3R strategies. This experience can help ensure measurable results for the 3R-Center by focusing on the initiatives that are most likely to benefit laboratory animals in Denmark. Jan sees the 3R-Center's mission as: enhanced cooperation in the field of the 3Rs; improved communication of the 3R area; increased 3R research in areas that can be put to immediate use; documentable results (in the short and long term).

ERWIN L ROGGEN

Erwin L Roggen holds a PhD in biochemistry and is the founder of the company 3Rs Management and Consulting ApS. Erwin has almost 25 years' experience of developing animal-free test methods and more than 10 years'

experience of implementing, using and validating these methods. Erwin's long-term 3R vision is for the industry and authorities alike to apply and accept testing methods and strategies that help safeguard health and safety without using animal models.

Erwin has a global network that actively drives the development, transfer and implementation of animal-free test methods as well as the application and validation of these methods among industrial players (product development) and the authorities (risk assessment).

As a goal for the 3R-Center's work, Erwin considers the prime target to be the establishment of a culture in which animal-free strategies are addressed first, and then refinement and reduction strategies when new products are tested, developed and marketed. The key words are collection and sharing of knowledge, sharing experiences and establishing the best 3R practices, communication, teaching/training and facilitation of the implementation, use and scientific validation of new methods within 3R strategies.

ADRIAN SMITH

Adrian Smith is a British veterinarian but has lived in Norway for the past 30 years. He has held a number of different positions in the Norwegian laboratory animal environment. Since 2007, he has served as the Secretary of Norecopa – the Norwegian national consensus platform for replacement, reduction and refinement of animal experiments (www.norecopa.no).

Adrian has gathered extensive knowledge of 3R resources through his work in the area and also has a good network. He has worked both as a researcher, an authority representative, head of an animal department and as disseminator of knowledge on alternatives, all of which enables him to see the issue from multiple angles.

According to Adrian Smith, the prime tasks for the 3R-Center are to quickly disseminate 3R advances within Denmark and abroad. Also, the mutual understanding, respect and knowledge-sharing between the various stakeholders must be increased. Adrian will also work for increased funding for this research area.

The Secretariat

- Tom Bengtsen, Head of Secretariat
(veterinarian)
- Louise Bjørn Brønden, academic officer
(veterinarian)
- Rasmus Normann Nielsen, communications officer
(MA in history and communication)
- Birgitte Vindahl Olsen, student assistant
(biology student)



The 3Rs – Replacement, Reduction and Refinement

William Russell and Rex Burch were the authors of the concept of the 3Rs, which they described in their book *The Principles of Humane Experimental Technique* in 1959. The increasing use of laboratory animals in research made it necessary to focus on animal welfare and the ethical issues involved in subjecting them to pain and related stress.

The wish to enable the research community to collectively follow certain guidelines to reduce the number of laboratory animals and alleviate animal suffering became Russell and Burch's 3R concept: Replacement, Reduction and Refinement. The following are the definitions of the 3Rs developed by the board of the Danish 3R-Center, with inspiration from Russell and Burch.

REPLACEMENT

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

REDUCTION

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

Reduction comprises instances where a smaller number of animals can be used in a given situation in a specific experimental model than previously. Consequently, reduction must always be a target for lowering the number of animals used for generating a certain amount of knowledge and not as a target for whether a given organisation, state or company has reduced the overall number of animals within a given time frame compared to corresponding previous periods of time. The development of animal models with a view to enhancing the scientific results achievable from each individual animal is therefore

also considered reduction. Reduction can be obtained using measures such as screening with animal-free models or technologies prior to animal testing, by using animals with the exact characteristics that one is interested in or by designing more systematic experiments.

REFINEMENT

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

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

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

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, LEO Pharma, Lundbeck and Novo Nordisk. The Danish 3R-Center works to promote the 3Rs in Denmark to focus on alternatives to animal testing and create even better conditions for laboratory animals.

THE DANISH ANIMAL WELFARE SOCIETY

“The Danish Animal Welfare Society (DAWS) believes that the use of laboratory animals should be minimised. 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 realising DAWS’ ambition to phase out the use of laboratory animals.”

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 to develop 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 optimise 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 drugs that are 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 obtain these goals and therefore also actively supports the Danish 3R-Center.”

The Danish 3R-Center
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