2018

ANNUAL REPORT

DANISH 3R-CENTER





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2018 was an eventful year for the Danish 3R-Center. Several activities are becoming firm traditions that we of the board and secretariat know and manage well by now - and for which you may know the Center.

The international symposium attracted a record number of participants this year and was the venue of many interesting discussions. We presented the 3R Award to Birgitte Kousholt who is a very deserving recipient exemplified by her focus on systematic reviews in her research at Aarhus University. You can read more about this in the annual report.

Another recurrent event is the annual allocation of research funding to the 3R area where we are always looking forward to reading the applications. Please keep submitting them. You can also learn more about the latest projects in the annual report.

The board has also worked on other issues. We discussed the number of laboratory animals in Denmark. The number of laboratory animals is often used in isolation as a parameter for the strength of the 3R environment and to indicate whether developments are going in the right direction towards reducing the use of laboratory animals. For this reason, the Danish 3R-Center has drawn up a statement paper to explain that it is not necessarily negative to have more laboratory animals. I hope you will read this with interest.

To give an impression of 3R developments in both Denmark and abroad, the annual report describes some of the events attended by the Danish 3R-Center, and you can also read about the events held at the Danish 3R-Center.

I would like to take this opportunity to thank my fellow board members and the secretariat for their great dedication in 2018. It has been a pleasure working with you.

Last, but not least, I wish to express my gratitude to the Danish Animal Welfare Society, DOSO, LEO Pharma, Lundbeck, Novo Nordisk and the Ministry of Environment and Food for their support of the Danish 3R-Center in 2018. This support enables us to continue our efforts to create a leading environment for the dissemination of information and application of the 3Rs to benefit both research and the laboratory animals.

Best regards

Christine Nellemann

Chairwoman of the board of the Danish 3R-Center

PROLOGUE

A HIGHER NUMBER OF **LABORATORY ANIMALS** IN DENMARK IS NOT NECESSARILY NEGATIVE

Comments by the National Committee for the Protection of Animals used for Scientific Purposes/the Danish 3R-Center on the number of laboratory animals used nationwide

It is in the interest of the National Committee for the Protection of Animals used for Scientific Purposes/the Danish 3R-Center that the discussion about animals used for scientific purposes is an informed one. Accordingly, we give high priority to disseminating knowledge about laboratory animals and alternatives to them in our daily work. In connection with the current publication of the number of laboratory animals being used nationwide, the Committee would therefore like to comment on the figure as it is necessary to be aware of a number of underlying matters to understand it.

It often attracts attention when the number of laboratory animals used nationwide rises or falls. Increases are typically considered negative and decreases are considered positive. Things are not that simple in our opinion.

If we start by looking at the number of animals used in the period from 2009 to 2012 (fig. 1), we can see that the number has declined from 300,000 animals in 2009 to 250,000 animals in 2012. The decrease is almost solely attributable to two companies giving lower priority to their research activities (the companies are protected by anonymity, but their identity is known to us).



Fig. 1: The number of laboratory animals in Denmark from 2009 to 2017

In the period after 2012, two years stand out in particular, i.e. the decrease in 2014 and the increase in 2016.

The reason for the decrease in 2014 is attributable to changed reporting rules1 and the number (198,980) is consequently not comparable to the numbers in the preceding or the subsequent years. This means that the decrease is not due to research-based initiatives to reduce the number of animals used which would otherwise be positive, of course.

The 2016 increase is due to successful research relating to preserving eels as a species in the future. Scientists succeeded in breeding eels beyond the age of the larval stage which, unlike eel larvae, must be reported as research animals, resulting in the significant increase in the number of animals used.

Laboratory animals used in 2017

The number of laboratory animals used in 2017 is stated as 236,100, which means that the figure returned to the level at which it has been since 2012. With the above changes to the number of laboratory animals nationwide in mind, we can establish that the annual use of animals for scientific purposes in Denmark has stabilized at around 240,000-250,000 animals for many years.

Though the number is not decreasing, the area of laboratory animals sees improvements on a daily basis. Scientists are thus making great headway for the 3Rs, but local laboratory animal improvements in the fields of Replacement, Reduction and Refinement become invisible when merely looking at the overall number of animals used nationwide.

A good example is *Refinement* – improvements that do not appear from the number of animals used nationwide but can be crucial for the welfare of the animals used for scientific purposes. If the near future should reveal a significant change to the number of animals used nationwide, this change will most likely be due to lower or higher research priorities being set by companies or at national level. Any research-based initiatives to reduce the number of animals used (Replacement or Reduction) will not necessarily have a significant effect on the national number, as another company may assign higher priority to research or new research activities can be set up in Denmark which may obscure this decrease.

Similarly, a focus on rises and falls in the absolute numbers reported does not reflect Reduction improvements of specific trials where the improvements did not lead to the use of fewer animals but resulted in a larger amount of knowledge obtained by the use of the same number of animals. According to Russell and Burch, this optimization of specific trials can also be considered to constitute Reduction.

Alternatives

Years ago, many were optimistic about the perspectives of replacing laboratory animals with alternatives, thereby drastically decreasing the number of animals used. In reality, there was probably only basis for optimism in the field of statutory safety testing of chemicals and medicinal products where the same type of tests is made on new substances, but which only accounts for a small part of the animals used for scientific purposes in Denmark.

The development of alternatives in other fields of research, for instance in connection with basic biological research and development of new therapies which account for a large proportion of the use of animals, has proven more difficult. There are many reasons for this. There are simply certain issues that currently cannot be resolved without using animals because it is not technically feasible to answer the research questions posted without using animal models. So the issue is not a matter of a lack of will to introduce Replacement but rather a lack of the necessary knowledge at this moment in time.

In order to accelerate developments, it is important that established laboratory-animal scientists are encouraged to break with their deep-rooted habits and established tradition at the workplace so that the scientist does not automatically think in terms of animal models when setting out to resolve a research task, but seek alternative solutions from the outset. The National Committee for the Protection of Animals used for Scientific Purposes naturally has a goal of replacing animals with equally suitable or better alternatives, and the Committee is, of course, working to both raise awareness of already existing alternatives and allocate research funds to Replacement projects.

As the development of alternatives is progressing at a slower pace than desired and a replacement of the majority of laboratory animals is probably not possible until years into the future, the National Committee for the Protection of Animals used for Scientific Purposes considers it to be most correct to also focus greatly on the two other Rs (Reduction and Refinement) in its work so that the number animals that are necessary to use can be minimized while ensuring their well-being at the same time.

The reporting rules for animals used for scientific purposes were changed in 2014. The most significant change was the introduction of a particular focus on the distress of the individual animal and any harm. This required the user of the animal to set up a continuous evaluation of the distress experienced by the individual animal during the trial period. And you could not submit the information about the animal before the trial was completed and you had the complete overview of the total and maximum distress for the animal.

As a consequence, the user could only report the use of the animal in the year in which the trial involving the animal ended, and not as soon as the trial began, as was previously the case. This meant that animals were included in trials in 2014 which would previously have been reported in 2014 but were now not released from the trial until 2015. Consequently, they were not included in the count until 2015. This resulted in a decrease in the total number of animals used for scientific purposes in 2014. Only when the figure for 2015 was available did we have new data for a 12-month period that allowed us to compare the annual use of laboratory animals again.





RESEARCH PROJECTS

An important part of the Danish 3R-Center's work is to support 3R research projects as part of the annual granting of research funding. In 2018, the Danish 3R-Center received 18 applications for support, of which the following three received a total of DKK 1.4 million.

RESEARCH GRANTS

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

PRIORITY IS GIVEN TO PROJECTS'

- → Quality
- → Implementability
- → Relevance

WHO IS ELIGIBLE TO APPLY FOR FUNDING?

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

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

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

RESEARCH PROJECTS SUPPORTED **IN 2018**

In 2018, the Danish 3R-Center received 18 applications for support, of which the following three received a total of DKK 1.4 million.



NEW ADVANCED **BLOOD INFECTION MODEL**

Thomas Emil Andersen, University of Southern Denmark

Blood infections are a major problem compromising around 12.000 Danish cases each year, of who around 2.000 die. Blood infections occur, when microorganisms spread from e.g. wounds or the urinary tract to the bloodstream, where the microorganism can cause a serious infection such as septic shock or endocarditis.

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

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

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

TRANSPORT AND METABOLISM OF AZOL ANTIFUNGAL DRUGS IN THE HUMAN TERM PLACENTA

Bjarne Styrishave, University of Copenhagen

Drugs are the group of chemicals that modern humans are by far most widely exposed to. Unfortunately, several drugs have the capacity to disrupt the endocrine system in humans unintentionally, for example by decreasing the production of sex steroids. The group of drugs known as azole fungicides is among the most endocrine disrupting drugs on the Danish marked. These drugs are first-line of treatment for fungal infections and are also widely used in pregnant women due to vulvovaginal candidiasis, infecting around 20% of all pregnant women.

The balance between male and female sex hormones during pregnancy is crucial for fetal development and significant efforts are made to investigate effects of drugs used during pregnancy on fetal sex development. Traditionally, these experiments are made with animals, most often pregnant mice as surrogate species. These animal studies suffer from two major drawbacks. First, the number of animals needed to conduct these experiments may be very high.

Secondly, data from such experiments may be difficult to extrapolate to human conditions due to differences in transport of drugs in the placenta from mother to fetus and due to differences in sex steroid metabolism. For example, the human placenta is able to transform

male sex steroids into female sex steroids, but the mice placenta is unable to do this. Consequently, the methods currently used to study effects of drugs on fetal development not only require a high number of experimental animals, but the relevance of the data obtained may be very limited for human pregnancies.

The present study funded by the Danish 3R Center proposes an alternative approach in which the human term placenta is used to study the transport and metabolism of azole antifungal drugs and sex steroids between mother and fetus. After giving birth to the placenta, both the maternal and the fetal circulation are reestablished. When azole antifungal drugs are added to the maternal circulation, the transport to fetal circulation, and the metabolism of azole antifungal drugs and sex hormones in the placenta can be analysed.

This approach will completely remove the need for conducting animal experiments, and at the same time provide data of high relevance to human pregnancies. The project will provide new information about azole antifungal drugs as endocrine disruptors and transport and effects during pregnancy. This, in turn, may lead to improved drug formulations and treatment regimes.



THE MOUSE PASSPORT

Axel Kornerup Hansen, University of Copenhagen

The mouse is the most widely applied experimental animal both in Denmark and globally. In diabetes research, which is one of the largest research areas in Denmark, mice are raised with a high fat diet that causes them to develop obesity and a type-2-diabetes-like condition.

How many animals to be used in each experiment are determined by how differently the mice reacts, and how fat or diabetic they become. Recent research has shown that this variation is largely controlled by the gut bacteria of the animals, which today, in contrast to previously, can be relatively easily characterized by a molecular examination of a fecal sample. Another influencing factor may be the animals' DNA.

Although very uniform inbred mice are used, newer methods have shown that the mice in their genetic material outside the actual genes mutate at high frequencies, and that these mutations control the variation of different expressions in the mouse. In this project, each mouse is characterized by their gut microbiota and their genetic material, after which an obesity test is performed with a well-known treatment.

It is determined how much of the variation in the results that can be described using these characteristics, and it is calculated how many animals to use, if such characteristics are available compared to the amount of animals to be used the way experiments are carried out today. In the long run, giving each mouse an individual characterization, a 'passport', will enable such studies with fewer mice, because it may be known which ones are the most or least susceptible to obesity and treatment, and if so it can be taken into account in the data evaluation, and eventually also be possible to select the most suitable mice for the study.

Supported 3R Projects

A list of all projects supported by the Danish 3R-Center can be found here:

en.3rcenter.dk/research/projects/



LABORATORY ANIMAL AND 3R EVENTS

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



WORLD DAY FOR LABORATORY ANIMALS 24 APRIL

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

This year, the World Day for Laboratory Animals was organized in collaboration between DOSO (the Cooperative Body of Danish Animal Welfare Organizations) and the Section for Experimental Animal Models (University of Copenhagen) which will also be the case in 2019.

Peter Mollerup, chairman of DOSO, gave a talk on the work of animal welfare associations/

DOSO on laboratory animal welfare in which he gave an account of the positive developments in the relationship between animal welfare associations and the world of laboratory animals in the past many years.

The relationship was quite strained years ago, and the parties were more confrontational. The animal welfare organizations fought for an immediate end to animal experimentation while the laboratory animal universe was characterized by an unapproachable and, according to Peter Mollerup, condescending attitude towards people outside the research environment.

The parties have moved closer since after realizing that both sides can benefit from dialogue and openness. Animal welfare organizations such as DOSO have moved from demanding an immediate ban on laboratory animals to promoting a long-term goal of avoiding the use of live animals for research purposes and that Replacement becomes the central R when setting research priorities. A result of this approach is that DOSO, like the Danish Animal Welfare Society, has chosen to support the work of the Danish 3R-Center. The world of laboratory animals increasingly considers animal welfare organizations an equal partner, a tangible result of which is the establishment of the 3R-Center.

Peter Mollerup acknowledged that major efforts are currently being made at all levels to fulfil the requirements of Reduction, Refinement and Replacement – for instance at the Animal Experimentation Council whose work Peter praised as serious and thorough. Peter emphasized that there is a good dialogue between animal welfare associations and the pharmaceutical industry which often hold meetings, conferences and field trips together. Peter expressed his wish to maintain this relationship which is quite unique in an international context.

The Danish 3R-Center/The National Committee for the Protection of Laboratory Animals used for Scientific Purposes were also represented in the programme, as board member Peter Bollen gave a talk on Animal welfare organizations and the efforts to promote "A Culture of Care".

In a laboratory animal facility, the Culture of Care concept involves defining the values and attitudes among the staff that are decisive for how they act and work together. A culture of care is manifested by a conscientious and respectful attitude and behaviour towards animals and motivates staff to accept and acknowledge their responsibility in every aspect of animal housing and use. A consequence of this culture is that encourages going beyond merely satisfying minimum statutory criteria.

If an animal facility just lives up to the legislation in the area, it does not necessarily ensure satisfactory animal welfare, care and use. Thus, it is important that all parties involved in both caring for and using laboratory animals are committed to the principles of 3R and that they must exhibit an appropriate, respectful and caring attitude towards the animals. Without this culture of care at the animal facility, it is unlikely to achieve sufficient optimization of both animal welfare and scientific results.





On behalf of the National Committee for the Protection of Laboratory Animals used for Scientific Purposes, Peter Bollen made the following appeal to animal welfare organizations:

- Animal welfare organizations are encouraged to know their organizational structure and thus the line of communication with management so that problems can be presented to the management when needed
- The animal welfare organizations are encouraged to contact the National Committee for the Protection of Laboratory Animals used for Scientific Purposes when in need of specific advice on the acquisition, breeding, housing, care and use of animals for experimentation and exchange of best practices
- Animal welfare organizations are encouraged to promote communications between the various specialized groups working with laboratory animals at facilities associated with the body
- Animal welfare organizations are encouraged to stay up-to-date with the laboratory animal legislation in force at any time
- The National Committee for the Protection of Laboratory Animals used for Scientific Purposes organizes an annual meeting for animal welfare organizations. Animal welfare organizations are encouraged to participate actively in this meeting – preferably with talks and other knowledge-sharing. In addition, animal welfare organizations are encouraged to obtain knowledge of technical and scientific developments within Replacement, Reduction and Refinement from other meetings and resources
- Animal welfare organizations are encouraged to be able to document their work on promoting Replacement, Reduction and Refinement at facilities associated with the body.
- Animal welfare organizations are encouraged to ensure that animals to be rehoused or returned to a habitat or production system are suitable for this purpose and that it is the best solution for the animal in terms of welfare.



INTERNATIONAL

CULTURE OF CARE NETWORK

At the FELASA congress in 2016, several presentations concerned the concept of *Culture of Care*, which made Thomas Bertelsen *(Senior laboratory animal veterinarian)* of Novo Nordisk propose the creation of a network that will actively work to spread the culture of care as this is essential in the efforts to secure ongoing improvements of laboratory animal welfare. The network was set up and currently counts 37 members from 16 different countries.

THE MEMBERS:

- Researchers, animal keepers / technicians and veterinarians.
- Animal welfare bodies and national committees
- Government representatives
- Communication experts
- Animal welfare organizations

The diverse group of members ensures the promotion of a welfare climate for both the animals and the people working with them.

The members are expected to work actively with and to promote a culture of care.

CARE TO JOIN?

Contact: Thomas Bertelsen (tsbt@novonordisk.com)

More information at https://norecopa.no/ more-re-sources/culture-of-care

Dorte Bratbo (University of Copenhagen) gave an interesting presentation entitled Improved pig and rodent welfare – effect of management method and training. The presentation left the impression of an animal facility which has embraced the idea of a culture of care to a great extent.

Dorte's presentation can be regarded as a departure from traditions and conventional thinking - and the idea of "why change something that works?"

Dorte argued that a whole-hearted effort to improve management and training methods not only results in improved animal welfare thereby improving the research results – but also in welfare improvements for the animal keepers whose work is naturally positively affected by dealing with more satisfied animals in their daily routines.

As can be seen, there are good reasons to seek to improve handling and training. Dorte presented a number of specific ways of improving welfare in pigs and rodents, respectively, by means of gentle handling, gentle procedures and reward-based training methods.

A good example of gentle handling is tunnel guiding of mice instead of tail handling - and tail handling may actually be a good example of an old habit. Tunnel guiding has several advantages, one of which is that the mouse's contact with the keeper/researcher is more voluntary, and the mice become less fearsome in behavioural tests, resulting in improved animal welfare and research findings.

Training of species such as pigs using clicker training can also improve animal welfare, but it is a training method that requires knowledge of the animals, time and creativity. A certain effort is required to attain the associated advantages. Clicker training aims to accustom the animals to everyday or testing procedures - preferably using food rewards to get the pig (or rodent) to work with rather than against the keeper/researcher.

It can be difficult to measure the effect of clicker training or gentler handling which is why you need to trust your perception. This is why trained/updated animal keepers/ researchers are essential in improving animal welfare.

Dorte's points about breaking with old habits for the sake of animal welfare and being willing to invest the time necessary to create a "working relationship" between keeper/ researcher and animals are at the very core of efforts to create a culture of care.

WORLD DAY FOR LABORATORY ANIMALS 2019

The theme of the World Day for Laboratory Animals on 24 April 2019 will be the significant benefit criterion (harm/benefit).

A mini seminar will be held in the afternoon, in continuation of the World Day for Laboratory Animals. The topic for the mini seminar will be animal testing and lifestyle diseases. Further information can be found on the web-

site of the Animal Experimentation Inspectorate (dyreforsoegstilsynet.dk)



SCANDINAVIAN SOCIETY FOR LABORATORY ANIMAL SCIENCE (SCAND-LAS) 26-28 APRIL

Representatives from the Danish 3R-Center attended Scand-LAS once again this year, and Adrian Smith, who is not only a board member of the Danish 3R-Center, but also secretary of Norecopa, helped organize the annual symposium which was held in Kristiansand.

The meeting topic was Beyond legislation -Best practice in animal research and offered 30 talks and five workshops (Applied genetics in laboratory mice; Environmental health monitoring; Harm-Benefit analysis – Current concepts and practical approaches; Recognition of pain and distress in laboratory rodents; Health monitoring).

Penny Hawkins of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) gave a thought-provoking presentation on *The 9 to* 5 rodent - Time for change? about the effect of light on mice and rats. About the effect of handling them and subjecting them to tests during their inactive phases and the effect of reversing the circadian rhythm to match that of the animals.

The Danish 3R-Center found the talk very relevant and we invited Penny Hawkins to give a similar presentation at the Danish 3R-Center's symposium in 2019.

Thomas Bertelsen of Novo Nordisk gave a presentation of how the company has worked proactively with animal welfare for more than twenty years which is well aligned with Thomas' work on spreading the knowledge of Culture of Care through the network mentioned above. Based on Novo's self-imposed bio-ethical obligations, Thomas gave examples of how Novo exceeds the statutory requirements of animal welfare – be it improved housing conditions, additional focus on training of animals and securing a uniform approach to the 3Rs, regardless of the company's global presence.

Per E. Ljung of the very young Swedish 3R center gave the presentation Barriers, behaviors and beliefs related to the 3Rs – a survey among researchers in Sweden. The Swedish center initiated the study to acquire knowledge of the "state of 3R" to become more familiar with its target group in its work to implement 3R.

As is the case in Denmark, Replacement is challenging to Swedish researchers in terms of practical implementation, and it would appear that the Danish and Swedish 3R centers are facing some of the same challenges.

Adrian Smith gave a talk on PREPARE which are guidelines for planning animal experiments. PREPARE means Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. Other guidelines, such as ARRIVE, are aimed at the reporting of animal experimentation when publishing the results.

The purpose of *PREPARE* is to help researchers improve the quality of their animal experimentation and to improve animal welfare at the same time. The *Danish 3R-Center* supports Adrian and *Norecopa* in their dissemination of PREPARE.

You can find *PREPARE* on *Norecopa's* website: norecopa.no/prepare/

Incidentally, it was at this year's Scand-LAS that the 3R-Center's representatives became aware of Stuart Ritchie who gave a talk on *Unconscious bias in scientific research.* They found both the speaker and the topic to be highly relevant and invited Stuart Ritchie to give the same talk at the Danish 3R-Center's symposium in November (read about Stuart's presentation later in this annual report under the description of the Danish 3R-Center's 2018 symposium).

One of the interesting workshops at the annual Scand-LAS was about recognizing pain in laboratory rodents, which requires both experience and training. The workshop included an interactive component with participants looking at pictures and video and using score sheets to indicate pain-associated behaviour.

SCANDINAVIAN SOCIETY FOR LABORATORY ANIMAL SCIENCE

Scand-LAS was founded in 1970, and as of today, the Society has around 350 members, mainly from the Nordic and Baltic countries. Our mission is to actively promote the understanding of the need for animals in research, the education of personnel involved in the use of laboratory animals, the accreditation of laboratory animal facilities and the development of Laboratory Animal Science (LAS) as a scientific discipline. Membership is open to everyone working within the field of LAS.

ANNUAL MEETING FOR ANIMAL WELFARE BODIES 7 JUNE

Each year, the National Committee for the Protection of Animals used for Scientific Purposes (UFA) 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 inspirational event offering networking for everyone with a professional interest in laboratory animals.

Christine Nellemann opened the meeting by welcoming the attendees and presenting the programme before talking about her work performed under the auspices of the National Committee for the Protection of Animals used for Scientific Purposes.

Christine also promoted six guidelines prepared by the Animal Experimentation Council on specific experimental procedures in regards to weight loss, blood sampling, fasting, dosing and tumour models, and five guidelines that are being prepared (Use of treadmill (mice and rats); Models for demyelinating diseases (such as sclerosis); Experiments on animals living in nature; Behavioural testing; Models for neuropsychiatric diseases).

GUIDELINES FOR SPECIFIC EXPERIMENTAL PROCEDURES

The Animal Experimentation Council has prepared a series of guidelines for specific experimental procedures in both Danish and English. You can find them on the website of the Danish Veterinary and Food Administration under The National Committee for the Protection of Animals used for Scientific Purposes.

In collaboration with both the Animal Experimentation Council and the animal welfare bodies, the National Committee for the Protection of Animals used for Scientific Purposes also prepared the document Responsibility when using animals for scientific purposes which aims to clarify the responsibilities of authorization holders and can also be found at the website of the Danish Veterinary and Food Administration.

THE DANISH NETWORK FOR ANIMAL WELFARE BODIES

- · Open to all members of Danish animal welfare bodies - regardless of education and position
- · Yet another possibility for knowledgesharing
- · Organizes knowledge-sharing meetings that are open to a larger group of members of animal welfare bodies
- · Can organize visits at the participants' facilities to demonstrate animal welfare initiatives
- · The members can contact each other with specific questions, etc.

Would you like to join?

Contact the secretariat for the National Committee for the Protection of Animals used for Scientific Purposes/The Danish 3R-Center to learn more about your options at: info@3rcenter.dk

At the annual meeting, the National Committee for the Protection of Animals used for Scientific Purposes focused on how the animal welfare body can promote, which is why Lisbeth E. Knudsen and Erwin Roggen, two of the Committee's other Replacement specialists, took the stage after Christine Nelleman to give a presentation on the topic, leading in to the day's workshop.

The about 100 representatives from most of Denmark's animal welfare bodies were divided into seven groups to discuss the topic. The workshop resulted in a number of interesting

input that can inspire the National Committee for the Protection of Animals used for Scientific Purposes / the Danish 3R-Center in the future work to raise more awareness and clarity about this complicated subject.

For the second consecutive year, the *National* Committee for the Protection of Animals used for Scientific Purposes presented their 3R award for animal technicians. The winner was Sara Mathez of Leo Pharma with her idea of using modified rabbit cages for rats which Sara tells more about on the next page.

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

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

ANIMAL WELFARE BODY The animal welfare body is tasked with the following:

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





THE WINNER OF THE 2018 3R AWARD FOR PARAVETERINARY STAFF, SARA MATHEZ (LEO PHARMA), WRITES:

Our rat cages are developed with a view to providing even more space than in the frequently used Type 4 cages — both with respect to area and height. We have therefore used rabbit cages with plexiglass sheets attached to the front of the cage to prevent the rats escaping through the cage bars (approx. price per cage DKK 3,000).

So far it is our experience that the rats not only seem to be calmer, but also exhibit better group behaviour and appear less stressed. We believe that the reason for the positive observations is that they have more space.

A simple reason for the improved group behaviour could be that we house five rats in each cage due to the increased floor space, compared to the above-mentioned Type 4 cages that only hold two or three rats. In addition, the rats are accessed through a hatch in one side of the cage, instead of from above, as in the Type 4 cages, which is probably less stressful as the rat's natural enemies would come from above.

Further studies need to be made of how the rats perform in behavioural tests after living in the new, larger cages, compared to the Type 4 cages.

WINNERS:

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

2018 Sara Mathez (Leo Pharma)

2019 ANNUAL MEETING

University of Copenhagen Frederiksberg Campus Bülowsvej 17 (the Lecture Hall), DK-1870 Frederiksberg C

(further details at the website of the Danish Veterinary and Food Administration - visit www.fvst. dk and search for the National Committee for the Protection of Animals used for Scientific Purposes).

3R competition for paraveterinary staff 2019

The National Committee for the Protection of Animals used for Scientific Purposes sponsors an annual competition where it is possible to win up to DKK 25,000 to initiate or try out a 3R initiative or 3R idea. Applicants fill out an application form in order to be considered for the award, following which the Committee decides which proposal(s) to support.

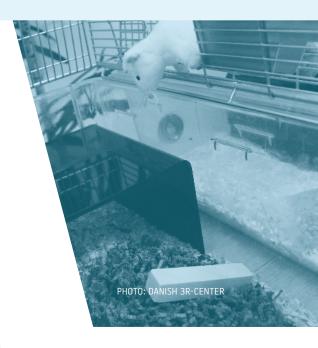
Send the application form to ufa-aarsmoede@ fvst.dk (further details on the website of the National Committee for the Protection of Animals used for Scientific Purposes).

The winner of the competition is presented during the open segment of the 2019 annual meeting for Animal Welfare Bodies.

The last part of the annual meeting was the so-called marketplace which is a significant reason behind the success of the Annual Meeting of the Animal Welfare Bodies. At the marketplace, individuals from various laboratory animal facilities bring one or more 3R initiatives, usually *Refinement*, from which they have learnt beneficial lessons. They present the improvements to the participants at the annual meeting to inspire them to take similar initiatives at their own facilities, and the participants can cooperate on further developing the ideas behind the 3R initiatives and thus facilitate further improvements.

3R initiatives for inspiration

3R initiatives presented at the Annual Meeting of the Animal Welfare Bodies can be found at the website of the National Committee for the Protection of Animals used for Scientific Purposes (fvst.dk) under the National Committee for the Protection of Animals used for Scientific Purposes.



SYMPOSIUM, FONDAZIONE GUIDO BERNADINI 28-29 JUNE

A significant reason why the Danish 3R-Center attends international events is that it gives us an opportunity to meet interesting speakers giving topical talks that we find relevant to present at our own annual symposium in Copenhagen.

This symposium in Italy under the heading of the three Rs in research project design: a prerequisite for good science featured excellent speakers with highly relevant presentations and we consequently invited no less than four of them to give presentations at the Danish 3R-Center's 2019 symposium.

These are the speakers that the symposium participants can look forward to experiencing: Natasha Karp: Sex bias in pre-clinical research; Helene Richter: Never replicate a successful experiment – facing the reproducibility crisis in the life sciences; Marco Annoni: Code of conduct for research integrity; Malcolm MacLeod: The way forward to implement the 3Rs in research - better science through implementation of the 3Rs.

An interesting presentation that also deserves mention was the talk on Transition to animal free innovations and animal welfare: Have the Dutch gone totally mad? by Jan-Bas Prins of Leiden University who based his lecture on a request from March 2016 by the then Dutch minister for agriculture, Martijn van Dam, for the Dutch national committee (NCad) to

prepare a schedule for phasing out animal testing before 2025. This spectacular initiative attracted massive attention in the scientific community, both positive and negative, and the initiative – and how to react to it – was discussed on several occasions by the Danish 3R-Center/National Committee for the Protection of Animals used for Scientific Purposes. Here, the initiative was definitely considered sympathetic, as a world free from laboratory animals is regarded as the ultimate goal, of course. However, it was not found expedient to set a deadline for such an initiative, and a 2025 deadline was also considered unrealistic.

Jan-Bas Prins stated that the Dutch ambitions had been rephrased from: The Netherlands should be world-leader in innovation without use of laboratory animals in 2025 to The Netherlands should be trendsetter in animal free innovations, which allowed the Dutch to accommodate the critics who found the ambition unrealistic as they believed it was counter-productive because it might risk overshadowing both real (minor) progress in the area and more realistic ambitions.

Jan Lund Ottesen, board member, gave the presentation The Danish 3R-Center - achievements 2013-2018 and future tasks.

EUROPEAN CONGRESS ON ALTERNATIVES TO ANIMAL **TESTING (EUSAAT)**

23-26 SEPTEMBER

The Danish 3R-Center was once more represented at EUSAAT which has developed into a congress that includes all three Rs. Accordingly, the conference was presented as follows: "The EUSAAT 2018 conference focuses on oral and poster sessions on Refinement and Reduction (e.g. animal welfare, education) as well as Replacement topics (e.g. disease models, non-animal tools for basic biomedical research, toxicity studies, omics techniques, advanced 3D models including recent progress in developing human-organ-chips)."

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. The conference attendants had quite varied professional backgrounds – from PhD students working with promising animal-free methods such as organ-on-a-chip and bioprinting to representatives of various companies and universities for whom the use of laboratory animals is still necessary.

It was evident at EUSAAT that the previously mentioned Culture of Care concept is definitely a significant concept in the world of laboratory animals in 2018, with both Susanna Louhimies of the European Commission, Thomas Bertelsen of Novo Nordisk and Thomas Lund of University of Copenhagen dealing with the subject in their own separate ways.

Susanna Louhimies talked about the importance of the individual animal welfare bodies working to promote a Culture of Care at individual animal facilities. She pointed out that it is only possible to create such a culture if the individual employee is committed to this. Not just the staff working directly with the animals, but also the management higher up in the system.

Thomas Bertelsen talked about the importance of obtaining information about your own institution's current culture of care in order to improve it further. Novo Nordisk has therefore developed a tool that can generate knowledge about this by means of a few questions.

Thomas Lund presented the results of a study called Different cultures of care for lab animals – a comparative study of practices at universities and private research institutions in Denmark which was carried out at the University of Copenhagen's Department of Food and Resource Economics. According to the study, there seem to be institutional differences between private-sector companies and the universities that affect the efforts to create a culture of care at the facility, as the first-mentioned institutions seem better equipped to implement initiatives supporting this work.

2018 was the year when EUSAAT took the initiative to attempt to establish organized collaboration between the European 3R centers.

There is a pleasing trend towards 3R centers being established in more and more European countries. The centers differ greatly in size,

format and affiliation, however. Some countries have centers that are affiliated with a public authority as is the case for the Danish 3R-Center, for instance. In other countries, the centers are integrated with universities. Some centers have almost no funding and base their work on volunteering. Other centers have many employees and allocate substantial funding for 3R research each year. Some centers cover all 3Rs while others only focus on Replacement. But the centers share a willingness and enthusiasm in relation to making a difference for the 3Rs and an interest in benefiting from knowledge and experience amassed by other 3R centers.

As an example, the annual EUSAAT conference had a session with presentations of the more recent 3R centers in Europe in particular and there was a panel discussion on the topic, moderated by Susanna Louhimies of the European Commission. In addition, the chairman of EUSAAT, Winfried Neuhaus, organized a non-public meeting for representatives of the 3R centers which the Danish 3R-Center also attended. At the meeting, there was a discussion of how new and existing centers could work together in future to share knowledge and experience.

The European 3R centers are very inclined to set up a more structured collaboration framework. EUSAAT will attempt to serve as the driving force in relation to securing a real establishment of the collaboration.

This will be followed up on in 2019 with detailed identification of the willingness and opportunities for the centers to work together in the future.

The initial plan is to describe each 3R center with information about the center's focus areas, competences and interests and what the center offers. The descriptions will contribute to identifying the potential collaboration and the form of knowledge that the centers can share with each other.

Incidentally, the initiative is well-aligned with the Danish 3R-Center's continual focus on international collaboration which the center has promoted since its establishment and which has resulted in various collaborations with Norecopa in particular.

EUSAAT 2019 WILL BE HELD ON 10-13 OCTOBER IN LINZ, AUSTRIA.





THE DANISH 3R-CENTER'S **SYMPOSIUM** 5-6 NOVEMBER

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

This year's symposium in Copenhagen was attended by more than 200 persons with an interest in the field of laboratory animal and alternatives (researchers, students, animal technicians and keepers, animal welfare organization representatives, etc.) which also indicates perhaps the most important reason for the symposium: gathering people to allow them to share ideas and network.

The participants were also able to take home various 3R related materials from the symposium such as grimace-scale-posters, PREPARE and ARRIVE guidelines, etc., which the Danish 3R-Center distributes on behalf of NC3Rs and Norecopa.





5 NOVEMBER

The year's moderator was Erwin Roggen, member of the board of the Danish 3R-Center. Erwin opened by introducing Joseph Garner of Stanford University as the first speaker at the symposium.

THERIOEPISTEMOLOGY — IMPROVING REPRODUCIBILITY AND TRANSLATABILITY

Joseph Garner, Stanford University, Department of Comparative Medicine, and by courtesy, Department of Psychiatry and Behavioral Sciences, Stanford, California, USA

Joe Garner based his presentation on the so-called reproducibility and translatability crisis that is now enjoying wider recognition than previously, according to the speaker. The wider recognition has facilitated a paradigm shift for the field of laboratory animal science which has moved from asking (at micro level)

"what have we controlled for in this (animal) model?" to "what have we chosen to ignore in this model, and at what cost?).

At a macro level, we are witnessing a shift from viewing animals as simple tools to increasingly considering them individuals. Joe Garner therefore believes that we are currently witnessing the birth of a new discipline that he chooses to term *Therioepistemology* – the study of how knowledge is gained from animal research.

In this context, Joe Garner pointed out that in spite of the above-mentioned crisis, abandoning animal testing altogether is not a solution because, if used "correctly", animal models still have tremendous research value.

Joe Garner presented a number of questions that can be used for critically evaluating (from a therioepistemological perspective) biomedical research based on animal models. In the speaker's view, the questions were well-suited for illustrating the broad reaches of the new discipline but added that they can eventually change or increase in number as and when the discipline evolves. Joe Garner also mentioned that, by formalizing the discipline, we can begin to discuss best practices that will improve the reproducibility and translatability of animal-based research with ultimate benefits for both people and animals.

Read more about *Therioepistemologi* in the paper *Introducing Therioepistemology: the study of how knowledge is gained from animal research.*

THE DANISH 3R-CENTER STATUS

Christine Nellemann, chairman of the board of the Danish 3R-Center

Christine Nellemann provided an update on the current situation for the Danish 3R-Center's work during its first five years of existence, describing the many initiatives of the center to promote the 3Rs in Denmark. Christine noted that as this year's symposium was the fifth of its kind, now was a good time to take stock of the situation.

Christine based her presentation on the four areas under which the center typically focuses its activities:

1. Collecting and disseminating information

It goes without saying that the website is essential in efforts to heighten awareness of laboratory animals and the 3Rs, not just to those working with research animals and alternatives, but the general public as well. The 3R-Center gives high priority to both target groups, and Christine mentioned that the website has seen a constant increase in visitors during its life.

The annual report is another key element of the communication work at the Danish 3R-Center. It is mainly aimed at those with a professional interest in lab animals and 3Rs. The annual report not only provides information on the current status of the Danish 3R-Center's work but also lends space to others working with research animals and alternatives to tell about their 3R efforts. The annual report is published in a print version in Danish which is distributed to the 3R-Center's stakeholders and at various 3R events and a digital version (pdf) in English which is available on the 3R-Center's website.

Christine also told about the 3R-Center's teaching materials about laboratory animals and the 3Rs which has been developed for use by lower-secondary grades 8-10, in biology classes and by biotech classes in upper secondary school. She mentioned that the upper-secondary teaching materials will be promoted at several "learning festivals" during 2019.

2. Initiating and supporting research

Christine stated that on behalf of the Minister for Environment and Food, the Danish 3R-Center distributes up to DKK 1.5 million to fund 3R research every year. So far, the 3R-Center has supported 16 projects within the areas of Replacement, Reduction and Refinement (see all projects at the 3R-Center's website).

The Danish 3R-Center has also initiated two 3R studies (The Danish 3R survey: knowledge, attitudes and experiences with the 3Rs among researchers involved in animal experiments in Denmark and Replacement in animal research – a qualitative study of barriers and opportunities) to achieve greater awareness of the prevalence of 3Rs in Denmark in order to also identify additional initiatives that the 3R-Center can launch to further increase the prevalence.

3. Hosting an annual international symposium

Christine stated that the year's symposium attracted a record number of attendants. In recent years, the 3R-Center has made an effort to attract more non-Danish delegates, which is starting to pay off as more than 30 overseas representatives attended this year's symposium. Christine noticed that the steadily rising attendance figures and the generally positive feedback were clear indications of the symposium's relevance.

4. International cooperation

The Danish 3R-Center has always given high priority to international cooperation which is why center representatives often attend international conferences, symposiums, network meetings, etc. Christine stated that board members are frequently asked to give presentations at such events, and the secretariat often participates with posters. Similarly, both the board and the secretariat work to establish contact with potential partners or speakers for our own symposium.

THE RELEVANCE OF 3R FOR THE ETHICAL EVALUATION OF ANIMAL RESEARCH

Peter Kunzmann, Institut für Tierhygiene, Tierschutz und Nutztierethologie (Stiftung Tierärztliche Hochschule Hannover)

In the satisfaction surveys sent out by the Danish 3R-Center after its annual symposium, several participants over the years have asked for an ethics presentation on animal research and the 3Rs, so the 3R-Center invited Peter Kunzmann, whom the 3R-Center had been recommended in connection with a conference earlier in the year.

In his presentation, Peter Kunzmann asked whether the 3Rs, notwithstanding the importance of the concept, represent the whole solution to the moral issues we are facing in connection with animal testing. Peter emphasized that the EU Directive (2010/63/EU) also articulates some of the animal-treatment allowances that must be made for moral reasons.

Thus, the directive states that animals have an intrinsic value that must be respected and the animals should always be treated as sentient creatures. Therefore, the use of animals in procedures should be restricted to areas which may ultimately benefit human or animal health, or the environment.

Based on the book Nuffield Council on Bioethics (2015), Peter Kunzmann presented different positions on animal testing, ranging from one extreme - that of the abolitionists -those seeking to ban animal research, to the more pragmatic attitudes relating to harm-benefit and moral dilemmas and, to the other extreme, those who believe that all animal testing is justified. In this respect, Peter made the point that the 3Rs cannot "satisfy" the abolitionists, though Replacement theoretically embodies the potential for this.

With respect to harm-benefit, Peter elaborated on the concept of harm, as it is difficult to measure in specific situations. And because procedures can never guarantee a positive outcome that can justify the harm caused, it is necessary to do everything possible to minimise it for moral reasons. So the 3Rs can satisfy the more pragmatic attitudes to animal testing.

Peter Kunzmann consequently attempted to take a closer look at the limitations of the 3R concept in a larger perspective. Peter made the point that even after having considered the 3Rs in connection with animal research, thereby minimising harm to the animals, the scientist must ask whether the animals' "loss of welfare" is, after all, defensible compared to the benefits gained from the study. It is therefore important to take a closer look at the extent of these gains and to establish criteria for the harm-benefit balance as the EU directive also requires.





TOY ANIMAL SIMULATORS FOR USE IN SMALL ANIMAL SURGICAL TRAINING

Rikke Langebæk, University of Copenhagen

Rikke Langebæk teaches surgery to veterinary students at the University of Copenhagen. In her work, Rikke uses modified toy animals which she continuously develops and refines. Thus the toy animals serve as an alternative to lab animals as the students can practice a vast number of surgical procedures on the modified toy animals before honing their surgical skills on live ones. Rikke also made the argument that learning was improved by practising on toy animals before training on live animals.

And these are not the only weighty reasons for using toy animals rather than lab animals in class. Rikke stated that the toy animals are not used for ethical reasons only. The use of laboratory animals in surgery classes is both cumbersome and expensive.

Therefore, ethical, practical and financial considerations led to increased focus on alternatives and thus to the establishment of a Surgical Skills Laboratory in 2007. The toy animals were developed for this laboratory for use in teaching basic surgery to fourth-year students. And the more than ten years of experience with the skills laboratory has shown that the toy animals are definitely a useful training tool as they allow the students to comfortably build confidence and security in surgical skills before practising on live animals.

Rikke also mentioned that the main components of the toy animals can be reused and their disposable elements are inexpensive and easily accessible, meaning that you obtain major positive benefits in the form of a reduced number of animals while also benefiting in terms of learning and ethics.

The following presentations from the symposium are not included here, as the projects are described in Part 1 (Research) in this annual report:

- · New advanced blood infection model (supported by the Danish 3R-Center in 2018) Thomas Emil Andersen (University of Southern Denmark)
- · Transport and metabolism of azol antifungal drugs in the human term placenta (supported by the Danish 3R-Center in 2018) Caroline Ødum (University of Copenhagen)
- · The mouse passport (supported by the Danish 3R-Center in 2018) Axel Kornerup Hansen (University of Copenhagen)

UNCONSCIOUS BIAS IN SCIENTIFIC RESEARCH

Stuart Ritchie, Social, Genetic & Developmental Psychiatry Centre, King's College London

As in Joe Garner's presentation, Stuart Ritchie based his contribution on the "reproducibility crisis".

Stuart presented a vast array of biases that can (unconsciously) enter all stages of a research process and thus impair or ruin the results and also render reproduction of the study impossible (see the list of biases in Stuart Ritchie's presentation here: www.3rcenter.dk/arrangementer/symposium-2018/).

Following up on this, Stuart Ritchie presented a number of examples of current initiatives from the recently established Open Science movement which aims to eliminate the bias issues.

Among the instruments are data sharing and pre-registration of studies, and the responsible researcher is also encouraged to follow a number of guidelines for conducting the research – all to increase the accessibility and transparency of the study, notably to enable verification of the studies.









ANIMAL-FREE SAFETY
ASSESSMENT OF CHEMICALS

TRANSITION TOWARDS

Anne Marie Vinggaard, The Technical University of Denmark

Anne Marie Vinggaard opened her lecture with a bit of background knowledge of the some 350 pesticides approved in the EU. She stated that the available knowledge of the potential harmful effects of the pesticides on the male reproduction organ is sparse, leaving an urgent need to develop new animal-free test strategies for use in the risk assessment of pesticides.

The development of the male reproduction system depends entirely on androgens such as testosterone, which is produced in the foetal testicles.

Chemicals that can interfere with the formation of these hormones or block the androgen receptors may cause genital abnormalities, impaired sperm quality or infertility — dysfunctions increasingly seen in the male population.

As indicated by the title of the presentation, Anne Marie and her colleagues are working on methods to assess the safety of these pesticides without the use of lab animals. Their strategy combines mechanistic data from human cells with computer-based and physiologically-based kinetic pesticide modelling. The computer models can predict the pesticide concentration in the foetus for a given dosage, and the cell models can predict the concentration in the foetus that will cause the harmful effects.

Anne Marie explained how the research group has been successful in predicting endocrine disrupting effects of a number of selected pesticides in males. They applied their method to twelve select pesticides out of which they chose to validate the alternative approach *in vivo* for six of the substances. They were subsequently able to conclude that their method has a long-term potential to reduce the use of animal testing in this area.

SPEED PRESENTATION OF POSTERS AND POSTER SESSION WITH WINE, SNACKS AND NETWORKING

After Anne Marie's talk followed a speed presentation of posters and a poster session. As a new feature for this year, participants who had brought posters were given the opportunity to give a two-minute speed presentation of them. This made it clearer for the symposium attendees to see who had contributed the individual posters so they knew who to contact with any questions.

The poster session doubled as a social event with wine and snacks, which many of the symposium visitors chose to attend. Seventeen posters were presented (also available on the Danish 3R-Center's website), and according to the subsequent satisfaction survey, the symposium visitors were very satisfied with this session's networking opportunities.

POSTERS AT THE SYMPOSIUM

(see all posters at the 3RCenter's website)

3R Centre Giessen – Interdisciplinary Centre for 3Rs in Animal Research

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

Charité 3R - the 3R Center of Charité Universitätsmedizin Berlin

Diabetes, blindness & Co: How to assess the welfare of genetically altered rodents?

Does relative humidity affect reproducibility of animal research?

Norecopa: a toolbox of online 3R resources

Maximising the research value of animals through collaboration and designing experiments around tissue reuse

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

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

Promoting tissue sharing

The Charles River Animal Model Evaluation **Program**

The Danish 3R-Center

The Danish 3R-Center – supported projects

The Danish National Committee for the Protection of Animals used for Scientific Purposes

The effect of relative humidity on water intake of C57BL/6J mice housed under conditions of controlled relative humidity at cage level

The Swedish 3Rs Center – a year in action

What can we do with experimental animals after the trial?







6 NOVEMBER

Erwin Roggen opened the second day of symposium with practical details and expressed his satisfaction with the great turnout before giving the floor to the first speaker of the day, Thomas Bøker Lund. Thomas' presentation is not included in this part, as the report on which his talk was based is described in Part 1.

DIRECTIVE 2010/63/EU -**FOCUS ON IMPLEMENTATION** AND THE THREE RS

Susanna Louhimies, European Commission

As the title indicates, the vantage point for Susanne Louhimies' presentation was the EU directive that aims to protect animals used for scientific purposes.

Susanna stated that the Commission had undertaken a detailed investigation of the effect of the directive among the member states. The survey showed that the advances in the area vary among countries, which is largely because the individual countries start at different levels.

There are certainly gratifying aspects in the field of experimental animals seen in a European perspective. Susanna mentioned the animal welfare bodies that contribute positively to animal welfare in many laboratory animal facilities while also contributing to raising both standards of research practice and an awareness of the 3Rs. In addition, animal

welfare bodies play an important role in the establishment of a culture of care in the individual animal facility, which is also an important element in efforts to generate consensus about the correlation between good animal welfare and good scientific practice.

Susanne also emphasized the importance of a strong National Committee for the Protection of Animals used for Scientific Purposes in the endeavours to promote the objectives of the directive which, like the animal welfare bodies, may suffer from insufficient expertise and underfunding. It is important for the national committees to get involved in the work of the animal welfare bodies and find a suitable practice in the related counselling.

Susanne also pointed out that it is important for everyone who plays a part in the sphere of laboratory animals in some way or another

(scientists, animal keepers/technicians, competent authorities (the Animal Experimentation Council/the Animal Experimentation Inspectorate), etc., to have knowledge about the 3Rs, which is why it is also worth considering from where and how this knowledge should be disseminated so that new knowledge continuously reaches relevant recipients. The 3R centers naturally play a part in this work and Susanna was therefore pleased with the increasing number of centers being established.

By way of conclusion, Susanna presented a number of current and future initiatives by the Commission to further expedite the objectives of the directive, including more training and education projects to equip future generations with the proper tools and the right attitude in relation to experimental animals.

Guidance from the EU Commission

Together with the member states, the European Commission has drawn up a number of guidance documents that delve into specific areas of the EU Directive on animals used for scientific purposes. The following guidelines are available on the Danish 3R-Center's website: Animal welfare bodies and national committees; Education and training framework; inspections and enforcement; Project evaluation and retrospective assessment; Severity assessment framework

www.3rcenter.dk/ressourcer/implementering-af-eu-direktiv-201063eu/





ANIMATCH – AN INNOVATIVE WEB-BASED PLATFORM TO SHARE ORGANS AND TISSUES

(WWW.ANIMATCH.EU)

Annemarie Lang, Charité Universitätsmedizin Berlin

Based on the EU Directive (2010/63/EU) which makes the following request: *Member States* shall facilitate, where appropriate, the establishment of programs for the sharing of organs and tissues of animals killed, Annemarie Lang spoke about *AniMatch*, which is an online service that enables researchers to connect with one another to share organs and tissues.

Annemarie stated that the number of animals used for scientific purposes has increased quite dramatically over the past many years. Not all animals are subject to actual animal testing. For instance, some animals are exclusively used by the scientist for the collection of tissues and organs for use in cell culture studies or in vitro models (animals killed for the purpose of extracting organs/tissues are not defined as experimental animals). Other animals are used for teaching purposes. In most cases, the scientist may only use one or two organs before discarding the rest of the animal. Annemarie stated that AniMatch has been established for the specific purpose of eliminating this waste.

There are quite weighty reasons why a tissue-sharing service makes sense – both in terms of ethics or money or a combination

of the two. Tissue-sharing can thus prevent many animals from being killed and there are financial benefits of receiving animals from a tissue-sharing service rather than purchasing live animals.

The tissue-sharing service also has the potential to become a significant resource bank for tissues and organs, enabling the scientist to get a large number of the required materials for research purposes and in principle, the service can give access to material from rare species.

Annemarie concluded her presentation by encouraging those interested to test the *AniMatch* demo (https://demo.animatch.eu/).

Tissue-sharing services

Based on Annemarie Lang's presentation, the *Danish 3R-Center* has collected links to a number of tissue-sharing services on the Center's website:

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

APPLICATION OF THE 3RS THROUGH POLICY, INSPECTION, AND PROJECT LICENCE ASSESSMENT

- EXAMPLES FROM THE UK REGULATOR

Peter Gray and Will Reynolds, UK Home Office Animals in Science Regulation Unit

This presentation was given by both Peter Gray and Will Reynolds who are Head of the Animals in Science Regulation Unit (ASRU) and Principal Inspector, ASRU, respectively.

Their talk presented a series of examples of how the British government and regulatory authority are working on different levels to ensure that the 3Rs are the foundation of all science involving the use of animals for scientific purposes.

Like Susanna Louhimies who stated that everyone who has a stake in the legislation or research relating to laboratory animals should have sufficient knowledge of the 3Rs, Peter and Will believed that the implementation of the 3Rs is everyone's responsibility.

Therefore it is not just the staff at the individual animal facility who are responsible for the implementation. The national governments and legislators should thus both inspire through their actions and provide ideal conditions for it. Governments can exercise great influence on the 3Rs when allocating research funds, which is a specific example of how governments/legislators can inspire through their actions.



MOUSE HANDLING MADE EASY

- REDUCING ANXIETY IN MICE AND THEIR HANDLERS

Jane Hurst, University of Liverpool

Jane Hurst of the University of Liverpool gave an interesting presentation on the handling of laboratory mice. This presentation appeared to be relevant to many, as mice are the most frequently used animal for scientific purposes and the mouse-handling methods presented by Jane are easy to implement at an animal facility.

Jane Hurst stated that her research group has demonstrated that the frequently used handling method of picking up a mouse by its tail adversely affects the animal, causing stress and anxiety in the mice that may consequently affect studies adversely.

Therefore, the research group has developed a number of improved, simple handling methods in which the mouse is picked up using what is known as a tunnel (e.g. a small plastic pipe) or a cupped hand, for instance. In her talk, Jane Hurst presented several different data sets behind the refined methods (*Refinement*) that all argued in favour of this being the future of mouse handling, and she gave tips on how to introduce the methods at an animal facility.

Note: More information is available at: www.nc3rs.org.uk/how-to-pick-up-a-mouse



PRESENTATION OF THE 3R AWARD



The next agenda item was the presentation of the year's 3R Award for the fifth time in the Center's existence. Erwin Roggen started by articulating the award winner's achievements which the board feels are exceptionally deserving of the award. Erwin was talking about Birgitte Kousholt – recipient of the Danish 3R-Center's 2018 award.

Birgitte has worked on implementing the 3Rs at institutional level ever since she was appointed designated veterinarian at the Department of Clinical Medicine at Aarhus University in 2013. And the board of the Danish 3R-Center has also paid attention to Birgitte's efforts ever since then.

The philosophy underlying Birgitte's work is to implement 3R improvements by both increasing the validity of the animal tests and the general knowledge of the science of laboratory animals.

In 2015, the award winner established August at Aarhus University together with Professor Gregers Wegener. August is a cross-disciplinary group working with systematic reviews in connection with pre-clinical animal testing. Systematic reviews have become an acknowledged 3R method, owing in no small part to Birgitte's contribution.

As chairman of the animal welfare body at the faculty in Aarhus, Birgitte has also had a decisive hand in developing the animal welfare body's overall strategic visions for the implementation of the 3Rs at the university.

Last, but not least, the award winner's interest in and great knowledge of laboratory animal research was emphasized, which has both

prompted the university to give priority to improvements among animal technicians, laboratory animal nurses and veterinarians which have proven important in the 3R work and given rise to several refinement and reduction initiatives that definitely contribute to optimizing the laboratory animal field at the university.

Erwin Roggen then asked Birgitte Kousholt to take the stage to accept the award. After the award presentation and a well-deserved round of applause, Birgitte told more about her work.

Winners of the 3R Award:

Ellen Margrethe Vestergaard The Danish Health Authority

Hanne Gamst-Andersen Novo Nordisk

2016

QSAR-teamet by Eva Bay Wedebye and Nikolai Georgiev Nikolov **Technical University of Denmark**

Grete Østergaard University of Copenhagen

2018

Birgitte Kousholt Aarhus University

THE 3R AWARD

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

The award is presented as part of the annual symposium.

For further details, go to:

www.3rcenter.dk/om-3r-centeret/3r-prisen/

THE DANISH 3R-CENTER'S 2019 SYMPOSIUM WILL BE HELD ON 12 AND 13 NOVEMBER AT CHARLOTTEHAVEN IN COPENHAGEN.

SURVEY OF SATISFACTION WITH THE SYMPOSIUM

A satisfaction survey was conducted among symposium participants, seventy of whom took the time to respond to the questionnaire, where each participant was asked to rate his/her level of satisfaction (or dissatisfaction) on a scale of 1 (dissatisfied) to 5 (very satisfied), which resulted in an average rating of 4.3.

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





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