



Front page photo: Tunnel handling for mice

PHOTO: THE DANISH 3R-CENTER

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Chairman's foreword

The Danish 3R-Center has entered its tenth year. The mission defined after the first board meeting in November 2013 was for the center to work on initiating applicable activities for immediate implementation of the 3Rs, create a forum for cooperation, discussion, exchange and dissemination of information about the 3Rs, and initiate specific research projects and recommend funding allocations by foundations for use in this field.

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PREF

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DENMARK'S 3R SYMPOSIUM 2022

Axel Kornerup Hansen, chairman of the board of the Danish

3R-Center, gave a presentation about the 3R-Center's work in 2022

The outlined vision was for Denmark to have a leading environment for the application and dissemination of the 3Rs.

The perception of the 3Rs changes over time. At the time of founding the 3R-Center, it was widely held that alternatives should replace specific experiments, but the board today views the entire animal-free research environment in Denmark as an important driver for visualizing the many possibilities for meeting research objectives without the use of animals. Throughout Denmark, laboratory animal facilities are working on improved and gentler animal experimentation and better housing facilities. And research funding from the 3R-Center and other foundations has helped develop methods to reduce the number of animals used for experiments.

We have not succeeded in reducing the small, but important number of animals in the severe category (severe suffering), but in our discussions with the groups of scientists that use the animal models with the highest severity, we can see that we share the ambition to reduce the number of these animals.

We also hope that our two clear statements on the use of animals for teaching and for production of antibodies will help reduce the use of animals for these purposes within a foreseeable timeframe.

The Danish 3R-Center has largely succeeded in creating a leading environment for the application and dissemination of the 3Rs, and the board consequently considers our vision from 2013 to be fulfilled. The current board, which will be resigning soon (31 March 2023) has therefore been considering a new vision for the Danish 3R-Center which may inspire the future work of a new board and of the laboratory-animal and alternatives environment in general. The original board which was re-appointed after four years is serving its tenth year, with the exception of a single member (Adrian Smith). Workload and a general election forced the Danish Parliament, the Folketing, to postpone the veterinary agreement that will secure the funding for a new four-year period, which means that the board's term was temporarily extended twice. However, new forces may now apply for seats on the board in 2023.

As the chairman of the board, I would like to thank the current board – including the now resigned member, Adrian Smith, and Birgitte Kousholt, his replacement – for their great effort during this period.

I would also like to thank our highly professional secretariat which has been instrumental in ensuring that we could do any work at all. I would also like to thank the entire 3R environment in Denmark for the large commitment shown in fulfilling the vision for the 3Rs.

Last, but not least, I would like to thank Peter Bie for contributing a lot of relevant input at the Danish 3R-Center's board meetings in his role as observer from the Animal Experiment Council.

I hope that you will find the annual report useful.

Axel Kornerup Hansen

Chairman of the board





RESEARCH

Research

An important part of the Danish 3R-Center's work is to provide financial support to 3R research projects on behalf of the Minister for Food.

Once a year, the Danish 3R-Center thus calls for applications for research projects that in one way or another have the potential to improve the laboratory animal area.

RESEARCH FUNDING

The Danish 3R-Center manages the allocation of DKK 1.5 million a year to support research in one or more of the 3Rs – *Replacement, Reduction and Refinement*.

Who is eligible to apply for funding?

Persons who are scientifically affiliated with an organization, institution or company in Denmark. It is possible to apply for up to DKK 500,000.

Research funding 2024

The call for the next round of applications for research funds for 2024 is planned to open in August 2023. Stay up to date about this on the Danish 3R-Center's website **en.3rcenter.dk** or sign up for the Danish 3R-Center's newsletter: **en.3rcenter.dk/newsletter-subscription** Research projects supported by the Danish 3R-Center in 2022

The Danish 3R-Center received 18 applications for support, of which the following three received a total of DKK 1.5 million.





Use of ultrasound for early diagnosis of necrotizing enterocolitis in laboratory premature piglets

Thomas Thymann / University of Copenhagen

Infants born prematurely have underdeveloped organs and require careful monitoring and support to ensure normal development. There is a high risk of developing necrotizing enterocolitis (NEC) in the intestines, which may be fatal, and which consequently requires keen attention of healthcare staff.

When NEC develops, air accumulates in the intestines as a consequence of intestinal bacteria fermentation. The accumulation of air expands the abdomen, which is one of the clinical signs of an advanced state. NEC is treated with analgesics and antibiotics but at worst may require surgical excision of parts of the intestines.

Intestinal accumulation of air – and the ensuing strain on the abdominal lining – is assumed to be associated with pain. In paediatric cases, medicine is used to relieve pain while it is a culling criterion for laboratory animals.

NEC research predominantly uses rodent models, but these models are significantly restricted by the size of the animals which makes it difficult to follow disease development and thus ensure timely intervention. Piglets born prematurely by caesarean section exhibit many of the same symptoms as children and are an acknowledged NEC research animal model. The University of Copenhagen has used it for more than 20 years. With the support from the Danish 3R-Center, we want to further develop and optimize the use of routine abdominal ultrasound to assess the stage of advancement of the condition.

The project aims to provide us with clear ultrasound-based criteria for timely culling so as to minimize pain and discomfort and keep this at an acceptable level in accordance with the animal experimentation permit.

> READ MORE ON EN.3RCENTER.DK

Did you know that...?

The Danish 3R-Center has supported a total of 28 projects since it was founded. You can find a list of all the projects in the appendix of the annual report, and further details about the projects are available on the 3R-Center's website: **en.3rcenter.dk/research/projects** 9

DENMARK'S 3R SYMPOSIUM 2022

Rie Vinggaard of the Technical University of Denmark – and last year's recipient of the 3R Prize – presented the project *The PluriLum assay: A novel stem cell-based assay for testing of chemicals' embryotoxic effects*, for which she received support from the Danish 3R-Center. Read about the project below.



PluriLum assay: A novel stem-cell-based assay for testing of chemicals' embryotoxic effects

Rie Vinggaard / Technical University of Denmark

For scientific, financial and ethical reasons, there is a need to develop new human and physiologically relevant methods for testing chemicals for developmental toxicity. This area makes up the majority of laboratory animals used for risk assessment testing of industrial chemicals.

We have developed a testing method based on human induced pluripotent stem cells – PluriLum – which we have reason to believe could become an important component of a future testing strategy for developmental toxicity. The method is based on three-dimensional cultures of aggregated stem cells, so-called embryoid bodies that mimic the very early human embryo formed four days after the conception of the embryo. These embryoid bodies are differentiated into beating heart cells and can detect teratogenic effects of known teratogens such as thalidomide, epoxiconazole and fluorinated chemicals. We have genetically modified the stem cells to make the activity of the differentiation marker NKX2.5 easy to read by luminescence.

We wish to determine and delimit the scope of the PluriLum assay by testing a broad panel of positive and negative teratogens and comparing the achieved effects with those seen in laboratory animals and other cell models. We also aim to illustrate the mechanisms of action of thalidomide and valproate for which we have RNAseq data awaiting bioinformatic analysis.

The project will contribute to further assessing the potential for the PluriLum assay in a future testing strategy for teratogenic effects of chemicals.

Replacement of meningioma animal models with a meningioma ex vivo/organoid model to test pharmacological advances in meningioma treatment

Mikkel Schou Andersen / University of Southern Denmark

Benign connective tissue tumours (meningioma) deriving from the dura mater on the surface of the brain occur frequently. Tumours in near proximity to vital structures such as the brainstem, large blood vessels or in surgically less accessible areas on the cranial base are often difficult to remove completely. Meningiomas are most often treated surgically or, alternatively, by radiotherapy, but both strategies are associated with a substantial risk profile.

Meningiomas that are not completely removed have a 20-44% chance of tumour regrowth. Medical treatment options have been tested since the 1980s with limited success and there is currently no effective medical treatment.

Meningiomas often express progesterone receptors on the surface of their cells. Another type of tumour which resembles meningiomas is the benign muscle tumour leiomyoma which can grow in the uterus. This tumour depends on progesterone receptors for tumour development. Leiomyomas are treated with hormone-suppressing therapy, with the selective progesterone receptor modulator (SPRM) as a novel addition. These medicines have never been tested in meningiomas.

The study aims to use meninges tissue directly from patients, purify the tissue, and isolate the cells, thereby culturing them in new tumours (organoids) in the laboratory. We will perform histology/ microscopy, DNA methylation and protein analysis of the organoids throughout to examine how they develop compared to the original tumour. We will then carry out trials of medicines on the organoids, using clinically relevant doses to assess whether the cells perish or stop developing.

This will be directly applicable in the clinic for patients from whom total tumour removal is not possible. This will enable us to use the organoids to directly examine the efficacy of medicines on tumour development.





DENMARK'S **3R SYMPOSIUM**

Mikkel Schou Andersen, University of Southern Denmark, gave the presentation Replacement of meningioma animal models with a meningioma ex vivo/organoid model to test pharmacological advances in meningioma treatment, which is a project for which he obtained funding from the Danish 3R-Center. Read about the project on this page.

The Danish 3R-Center's focus on severe suffering

For several years, the Danish 3R-Center has focused keenly on animal models in the severe category to stay up to date on the area and discuss with those conducting the experiments the possibilities of reducing the severity for the animals.

In 2022, the Danish 3R-Center published a brief report on this topic (Experimental animals with the highest degree of suffering in 2021). The report concluded that there is no reason to assume that the Danish use of laboratory animals in the severe category will sharply drop in the short term, and



interviews with users of the high-severity models also gave the impression that such experiments will only be allowed in Denmark after meticulous case processing at the Animal Experiments Inspectorate and the Animal Experiment Council.

However, the 3R-Center found it probable that it might have a positive impact on laboratory animals to establish contact and facilitate sharing experiences between different researcher groups that use animal models with the same degree of severity.

The use of animals in the severe category can be categorised into two subgroups:

- Disease models for especially painful conditions in humans
- Statutory investigations with unknown endpoints

A large part of the former group concerns mice which are used to study multiple sclerosis using EAE (Experimental Autoimmune Encephalitis) models. Based on the report, the board decided to host a meeting for scientists using this model notably to give the scientists an opportunity to share experiences about the model and draft a consensus paper. One of the intended outcomes was to reach an agreement on how severe symptoms the animals must experience to meet the specific research projects' purposes and to recommend best practices for Refinement measures.



Representatives of both the board and the secretariat held a very fruitful meeting with the scientists, and work is in progress on preparing the consensus paper.

A large part of the latter group concerns fish. International regulations (EU directives, medicinal product registration requirements in non-EU countries, etc.) may result in an obligation for Denmark to carry out animal experimentation where the endpoint of the experiment is not known in advance, which may result in severe suffering for the animals. New substances that may eventually potentially end up in our aquatic environment must be tested to see if they can cause disease and death in animals in the aquatic environment. Such testing involves fish, which are covered by the Animal Experimentation Act, and crustaceans, which are not.

It can be difficult to observe early symptoms of disease or injury in fish, causing a number of fish to die during these experiments if the substance examined is indeed harmful.

The current board therefore encourages the coming board to establish contact between scientists conducting such investigations in fish to also enable them to share experiences and discuss the possibility of improving the models to reduce the severity for fish as much as possible. The board generally also encourages the coming board to maintain focus on animal models in the severe category. ESEARCH

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DISSEMINATION

Dissemination of information

Dissemination of information has always been key to the Danish 3R-Center's work. Not only to individuals with a professional interest in laboratory animals and alternatives, but also to interested private individuals as well. The Danish 3R-Center believes that knowledge about the use of laboratory animals is fundamental for being able to discuss the matter on an informed basis in society.

The Danish 3R-Center's symposium

The largest individual event for the Danish 3R-Center is the annual, international 3R symposium. The symposium is an opportunity for the Danish 3R-Center to tell about its work over the year and gives researchers financially supported

READ MORE ON EN.3RCENTER.DK

You can find the programme, presentations and posters from this year's symposium at the 3RCenter's website: en.3rcenter.dk/symposium/ symposium-2022 by the centre a platform for disseminating their 3R project results to the laboratory animal environment. The Danish 3R-Center also invites national and international capacities with 3R expertise to talk about their respective spheres of work.

Each year, around 175 persons with a professional interest in laboratory animals and alternatives participate in the symposium, which means that a significant segment of the 3R environment is present, providing a unique opportunity for peer networking and mutual inspiration.

At the year's symposium, the participants benefited from not only excellent networking possibilities, but also no less than sixteen presentations and a panel discussion about a future without laboratory animals.





DENMARK'S 3R SYMPOSIUM 2022

Julián Albarrán Juárez, Aarhus University, presented the results of his project Establishment of an in vitro model to investigate extracellular matrix and vascular mechanical interactions in human arterial disease, for which he received support from the Danish 3R-Center.



DENMARK'S 3R SYMPOSIUM 2022

Matheiu Vinken, Vrije Universiteit Brussel, Belgium, gave the presentation The European ONTOX project: safer chemicals using less animals.



Symposium satisfaction survey

Around 100 symposium participants were surveyed in the days after the symposium to determine their level of satisfaction with the event. Each participant was asked to rate his/her satisfaction (or lack thereof) on a scale of 1 (dissatisfied) to 5 (very satisfied). This resulted in a high average rating of 4.5.

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

Note

The Danish 3R-Center's 2023 Symposium will be held on 6 and 7 November at Radisson Blue, Amager, Copenhagen.

Average satisfaction



The 3R Prize **Mette Gjerskov of the Danish Parliament (the Folketing)** won the 2022 3R Prize

The Danish 3R-Center has awarded a 3R Prize at each annual symposium since 2014. Mette Gjerskov, this year's winner, was unable to attend the symposium due to government negotiations following the general election on 1 November. Mette Gjerskov will be presented with the prize at a later occasion.

The board decided to award the prize to Mette Gjerskov based on her resolve as Minister for Food when the Danish 3R-Center was established in 2013.

Mette Gjerskov was a strong proponent of the responsibility of the industry, authorities and universities for ensuring the best possible treatment of Denmark's laboratory animals and for working to minimize the number of animals in experiments, for instance by means of methods to replace the use of animals.

In the period leading up to the establishment of the Danish 3R-Center, Mette Gjerskov brought together stakeholders in the area, including animal welfare organizations, to discuss the options and needs in relation to improving the laboratory animal area.

Mette Gjerskov succeeded in securing funds to partially finance the Danish 3R-Center and established the framework for the ministry, animal welfare organizations and the industry to join forces in financially supporting the 3R-Center. This required a visionary minister, indeed.

The board would therefore like to take this opportunity to congratulate Mette Gjerskov on her highly deserved 3R Prize.



Winners of the

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Samantha Saunders, Cruelty Free International, gave the presentation Reducing and replacing animal experiments: Europe needs an action plan.



DENMARK'S **3R SYMPOSIUM** 2022

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Herwig Grimm, University of Veterinary Medicine Vienna, gave the presentation A sheep in wolf's clothing: Why does the harm-benefit analysis not get grip in animal research?

DISSEMINATION

The Danish 3R-Center's website



Did you know that...?

The Danish 3R-Center published more than 450 news items from the 3R world on its Danish and English website in 2022.

The website has seen a constant increase in visitors since its establishment in 2014.

The website is the Danish 3R-Center's most important tool for disseminating knowledge about the 3Rs and informing the laboratory animal and alternatives environment about relevant events.

Another important task managed by the Danish 3R-Center is the dissemination of knowledge about laboratory animals to interested individuals, including schoolchildren. Much of the website's content is intended to serve this purpose. At the website, visitors can find information about experiment targets, the number and species of laboratory animals used.

The Danish 3R-Center's website

Researchers

News from the 3R world

Information about relevant events (symposiums, mini-seminars, animal welfare bodies' annual meetings, etc.) **Newsletters Research funds Research projects** The 3R Prize **Resources** for improving your research (e.g. PREPARE) Articles about laboratory animals and animal testing **Presentations** from 3R symposiums organized by the 3R-Center **Guidelines** for implementing EU Directive 2010/63/EU Links to teaching resources (for future users of laboratory animals)

Links to tissue-sharing services Annual reports Statements and recommendations

Individuals/schoolchildren

Teaching materials on laboratory animals and the 3Rs

Factual and basic information about laboratory animals

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

Politicians and stakeholders

Goals and goal-achievement forms for the Danish 3R-Center Minutes of board meetings Annual reports



DENMARK'S 3R SYMPOSIUM 2022

Tobias Wang, Aarhus University, gave the presentation *Perception in pain in fish and other "cold-blooded animals*".



Article on the use of animals in teaching

For its website, the Danish 3R-Center has written a long-form article about the laboratory animals used for teaching. After the 3R-Center in 2021 published its statement "Use of animals in teaching", the decision was made to prepare and publish two articles on the website about the permit-based use of animals as well as the non-permit-based use of animals to give the public insight into the purpose of these uses.

The first article, which has now been published on the website, deals with permit-based use of laboratory animals in teaching. READ MORE ON EN.3RCENTER.DK

Laboratory animals in teaching (permit-based use)

You can find the article (in Danish) on the Danish 3R-Center's website: **3rcenter.dk/ressourcer/artiklerom-forsoegsdyr-og-dyreforsoeg**

Participation in national and international events

Since the Danish 3R-Center was established in 2013, it has focused on raising the profile of the center's work at national and international events alike, which was also the case in 2022.

FELASA

The Danish 3R-Center took part in FELASA (Federation of European Laboratory Animal Science Associations), which was held on 13-16 June in Marseille. The conference theme was "Communication in Animal Research", which was an interesting approach to the subject of laboratory animals. The secretariat of the Danish 3R-Center and the Animal Experiments Inspectorate, which work closely together in their daily activities, had prepared a joint poster to illustrate the communicative benefits of Denmark's decision on a shared location for the secretariats of the Danish 3R-Center, the National Committee for the Protection of Animals used for Scientific Purposes and the Animal Experiment Council (Animal Experiments Inspectorate) which enables the secretariat staff to share knowledge and coordinate communication tasks on a daily basis.

> The Danish 3R-Center had brought a poster to FELASA to illustrate the communicative benefits of Denmark's decision on a shared location for the secretariats of the Danish 3R-Center, the National Committee for the Protection of Animals used for Scientific Purposes and the Animal Experiment Council (Animal Experiments Inspectorate).



Danish Cancer Research Days

The Danish Cancer Research Days took place 25-26 August 2022. The Cancer Research Days is a forum for bringing together researchers, patient representatives, healthcare professionals from many disciplines and decision-makers in the healthcare sector. The event features short presentations about new research, therapies and socio-economic matters relating to cancer.

The Danish 3R-Center was represented with a stand at the conference to inform cancer researchers working with laboratory animals about the 3R-Center's research funding in particular. It is also important for the Danish 3R-Center to participate in other conferences than laboratory-animals conferences *per se* in order to reach scientists that usually will not attend such conferences.

EUSAAT

On 26-28 September, representatives of the Danish 3R-Center once again participated in the EUSAAT conference (European Society for Alternatives to Animal Testing) which is held annually in Linz, apart from every third year when there is a world congress (World Congress on Alternatives and Animal Use in the Life Sciences).

The three-day conference attracted researchers, students, welfare organizations and authorities from many countries. The broadly based programme covered all aspects of the 3Rs from the point of view of research, NGOs and authorities.

Danish Veterinarians' Annual Meeting

The Danish 3R-Center was represented with a stand at the annual meeting of the Danish Veterinary Association (DDD) on 7-8 October. The annual meeting of veterinarians was held in combination with the annual meeting of veterinary nurses.

The Danish Veterinary Association invited the 3R-Center to raise awareness that the Veterinary Association also has veterinarians in the field of life sciences.

The 3R-Center's stand was popular, particularly on the second day of the annual meeting when veterinary students and nurses were also present.

EPAA

On 15 November, the secretariat participated in the EPAA meeting in Brussels. EPAA (European Partnership for Alternative Approaches to Animal Testing) is a partnership between industry, authorities and animal welfare organizations. Its purpose is to gather and share knowledge and resources to accelerate the development, validation and approval of alternatives at national, European and global level.

Promotion of teaching materials

A few years ago, the Danish 3R-Center had teaching materials made about laboratory animals and the 3Rs – for both lower and upper secondary school students – and in 2022, the materials were promoted at the education fairs Lærfest in Copenhagen and Århus and the BigBang festival in Odense. These events target teachers and educators.



INTERNATIONAL COOPERATION



International cooperation

Since the establishment of the Danish 3R-Center, it has been important for both the board and the secretariat to raise the center's profile in the international 3R environment to improve the possibilities of setting up cooperation with foreign 3R organizations.

NOTE!

12th World Congress on Alternatives and Animal Use in the Life Sciences

The World Congress will be held on 27–31 August at Niagara Falls, Canada.

www.wc12canada.org

Undoubtedly, the Danish 3R-Center has managed to raise awareness about its existence, which has contributed to the 3R-Center becoming a relevant partner for 3R organizations outside Denmark – including organizations with substantially higher budgets than the Danish 3R-Center, as illustrated in the example below. 25

Cooperation between six European 3R centres

THE DANISH 3R-CENTER / NC3RS / 3RS-CENTRE UTRECHT LIFE SCIENCES / THE SWEDISH 3RS CENTER / CHARITÉ 3R / SWISS 3R COMPETENCE CENTRE

A current collaboration worth mentioning is the cooperation with the above organizations. For many years now, the cooperation has proved effective and manoeuvrable.

The organizations hold monthly meetings to update each other on individual initiatives that could benefit the other organizations.

A prime purpose of the cooperation is to plan a joint webinar on animal-related topics for professionals with an interest in laboratory animals. In 2022, the planning started for a Culture of Care webinar which will be held on three Wednesdays in June 2023.

Mark the dates!

Culture of Care

Together with five other 3R centers, the Danish 3R Center organizes a webinar series about Culture of Care on 7, 14 and 21 June 2023.



DENMARK'S 3R SYMPOSIUM 2022

Jens Licthenberg, Novo Nordisk, gave the presentation *The ethical value of animals.*



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DENMARK'S 3R SYMPOSIUM 2022

Donald Ingber, the Wyss Institute at Harvard University, gave the (online) presentation *Recapitulating human biology, disease states, and therapeutic responses in vitro.*

European Network of 3R Centres (EU3Rnet)

Another initiative deserving mention is EU3Rnet, which is a network of European 3R organizations which saw the light of day in 2018. In a consensus statement issued in 2020, the individual members of the network commit to a serious dissemination effort on various digital platforms to increase the probability that important progress in the 3Rs will not remain a national matter only, but is also communicated internationally. During 2022, the network published two articles to which the Danish 3R-Center has also contributed. The two articles are published in Alternative to Laboratory Animals:

- The rise of Three Rs centres and platforms in Europe: pubmed.ncbi.nlm.nih.gov/35578444/
- The Current Status and Work of Three Rs Centres and Platforms in Europe: journals.sagepub. com/doi/ full/10.1177/02611929221140909



External contributions

In recent years, the Danish 3R-Center has given a number of external research institutions the opportunity to describe their 3R-related activities in our annual report to convey an impression of the widespread efforts in Denmark to improve the area of laboratory animals.

In recent years, the University of Aarhus, Novo Nordisk, the Technical University of Denmark, the University of Copenhagen, LEO Pharma, Lundbeck and the University of Southern Denmark have assisted in this.

In this year's annual report, the Danish Animal Welfare Society writes about their attitude to the use of laboratory animals.

The Danish Animal Welfare Society's attitude to laboratory animals

Anne Sofie Meilvang, Biologist and project manager for international projects, Danish Animal Welfare Society

The Danish Animal Welfare Society is Denmark's oldest animal welfare organization and works to improve conditions for laboratory animals and reduce their use. This has been a core issue for the Danish Animal Welfare Society for a great many years.

Back in 2001, we worked with Novo Nordisk to improve housing conditions for their animals, thereby improving their welfare. It was controversial for an animal welfare organization to join forces with a pharmaceutical company, but the top priority in this context was improving the conditions of animals used in experiments.

The Danish Animal Welfare Society also helped start the 3R-Center in 2013 and has supported the center ever since. The Danish 3R-Center is a result of unique cooperation between pharmaceuticals and animal welfare organizations, which underlines the pragmatic approach often pursued by the Danish Animal Welfare Society, focusing on dialogue and collaboration – despite differing viewpoints.

The Danish Animal Welfare Society's attitude to laboratory animals is that all research involving live animals should be replaced by research using alternative methods. However, we acknowledge that this is not possible in the short term and until it is, one of our goals is for the animal experimentation that is still being conducted to take place with as much concern for the animals involved as at all possible. We are also working to minimize the number of animals used per experiment and to prevent unnecessary experiments. We also want the housing and living conditions of laboratory animals deemed necessary for animal experimentation to meet the highest possible animal welfare standards.

This means that we both support the 3R principles and the 3R-Center, as we want to raise awareness

and implementation of the 3Rs in the public and private sectors and support 3R research.

We also want to achieve political support, both in Denmark and the EU, for laboratory animals and possibly tighter legislation where necessary and relevant.

Animal experimentation at educational institutions

Despite using relatively few animals – about 4,000 a year – at the educational institutions for teaching future veterinarians, physicians and biologists about physiology, anatomy and surgery, for instance, the Danish Animal Welfare Society believes that it is important for universities to stay keenly focused on the 3Rs. It should always be considered whether the animals can be replaced by alternatives, whether the number can be reduced and if the animals are housed in the best possible conditions and treated as gently as possible during the experiments.

The Danish Animal Welfare Society believes that it is essential for educational establishments to prioritize teaching about the 3Rs to give students the greatest possible respect for laboratory animals and safeguard that, after graduation, they will be aware of alternatives and give top priority to laboratory animal welfare.

We also work internationally

The Danish Animal Welfare Society is also involved with laboratory animal issues at international

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DENMARK'S 3R SYMPOSIUM 2022

Jennifer Rosowski and Stefan Hippenstiel, Charité in Berlin, together gave the presentation *Der Simulierte Mensch and the Charité 3R perspective.*

level. By way of example, we are represented in the Eurogroup for Animals' "Animals in Science", a group that also aims to ensure an ambitious implementation of the 3Rs with a final goal of full replacement of animals with more humane scientific approaches. Through Eurogroup for Animals, the Danish Animal Welfare Society last year encouraged our followers to sign a joint European initiative to end animal testing in the manufacture of cosmetics once and for all. This is because even though the EU in 2009 banned the use of animals for testing cosmetics, the experiments are governed by several pieces of legislation, which means that some animals are unfortunately still being used to test ingredients in cosmetics. We do not think this is right.

The petition was a great success, fortunately, as more than 1.2 million EU citizens supported the initiative. It also showed us that consumers still have a great desire to reduce the use of laboratory animals. We also closely monitor laboratory-animal developments further afield.

For instance, the Danish Animal Welfare Society supports the "The Long-Tailed Macaque Project". The long-tailed macaque (Macaca fascicularis) is a monkey that is frequently used as a laboratory animal. They are unfortunately often caught in the wild and 'laundered' by registering their place of birth as a breeding facility. Among the aims of the project is to ensure compliance with the applicable legislation and safeguard that longtailed macaques are not captured in the wild and sent to research facilities. The project also aims to improve housing conditions for laboratory monkeys. Though monkeys are no longer used for experimentation in Denmark, we believe that this is an important project to support, as we risk killing off the wild monkey populations and because the use of wild animals for experimentation entails a number of grave welfare issues.





The Danish National Committee for the Protection of Animals used for Scientific Purposes (the national committee)

In Denmark, we both have a 3R-Center and a national committee. As the seven members of the committee also function as the board of the Danish 3R-Center, it will probably be difficult for many to distinguish between the two initiatives, which is why this annual report will account for some of the work done under the auspices of the national committee.

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

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

The National Committee for the Protection of Animals used for Scientific Purposes works to promote the use of the 3R principles.

The committee is set up to advise the Animal Experiment Council/the Animal Experiments 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.

NOTE: THE ESTABLISHMENT OF A NATIONAL COMMITTEE IS CONSEQUENTLY MANDATED BY EU LAW, UNLIKE THE DANISH 3R-CENTER WHICH IS A NATIONAL INITIATIVE.

Annual Meeting of the Animal Welfare Bodies 2022

In Denmark, the Annual Meeting of the Animal Welfare Bodies is the national committee's most significant individual initiative – particularly in relation to positively impacting Denmark's animal welfare bodies – as the vast majority of Denmark's animal welfare bodies are represented at the meeting.

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The Annual Meeting of the Animal Welfare Bodies is a forum for lectures, workshops and networking – primarily centred on laboratory animal welfare. With the majority of the animal welfare bodies being represented, the annual meeting provides a good opportunity for disseminating information from the national committee and knowledge from presenters and the workshop into Denmark's laboratory animal facilities In recent years, the annual meeting has also included a marketplace where meeting attendants can inspire each other by presenting new approaches and methods that have improved conditions for laboratory animals at their respective workplaces.

The theme of the 2022 Annual Meeting of the Animal Welfare Bodies was "Reduction of the impact on animals in experiments", which was both reflected in the presentations and the traditional workshop at the annual meeting where participants first discussed the theme of the day in groups and then in a plenary session.



Advising the Animal Experiment Council

Meeting of the Animal Experiment Council The council is represented at the 3R-Center's board meetings with one member who has observer status. The gentleman in the foreground is Peter Bie, who was the observer in the period 2013-2022, after which he was replaced by Tobias Wang.

The National Committee for the Protection of Animals used for Scientific Purposes is also tasked with giving advice to the Animal Experiment Council/the Animal Experiments Inspectorate in matters concerning the acquisition, breeding, accommodation, care and use of laboratory animals and safeguarding exchange of best practices (pursuant to Executive Order No. 556 of 30 May 2013).

In 2022, the Animal Experiment Council sought advice from the committee in connection with pain relief for neonatal mice and rats and the use of tunnels as an alternative hiding place. In recent years, the national committee has worked to raise awareness of the advisory offerings available to the Animal Experiment Council from the committee and to enhance communication between the national committee and the Animal Experiment Council. Since the establishment of the 3R-Center and the national committee in 2013, the Animal Experiment Council has been represented by an observer at the meetings of the Danish 3R-Center/the national committee, making it obvious to further strengthen the collaboration with the observer, and thus the cooperation between the committee and the Animal Experiment Council.

DCASTING CORPORATION



DENMARK'S 3R SYMPOSIUM 2022

Janne Koch, LEO Pharma, gave the presentation *Thoughts on a future* without laboratory animals.



The actions of animal welfare bodies during the Covid lockdown

In 2021, the National Committee for the Protection of Animals Used for Scientific Purposes decided to investigate how Denmark's laboratory animal institutions had dealt with the pandemic.

The committee prepared a questionnaire with 13 questions about their actions when the crisis started and during the lockdown period as well as about takeaways for the future.

The questionnaire was distributed to Denmark's then 47 animal welfare bodies, 36 of whom answered the questions.

The survey formed the basis for a report which aimed to highlight the most important aspects of the responses, discuss them and single out various internal measures that may inspire other institutions in comparable future crises. The report concludes that while the pandemic's consequences were obviously predominantly negative, society – including laboratory animal institutions – learned valuable crisis-management lessons which could benefit the individual institutions going forward.

Another important point in the survey is that the pandemic not only provided the management with valuable information for dealing with future crises but inspired the laboratory animal institutions inspiration to take a different approach to everyday tasks.

Website en.natud.dk

By contrast with the Danish 3R-Center's website, which mainly addresses researchers and the general public, the committee's website is primarily aimed at Denmark's animal technicians. It gives them access to news within their work areas, and they can find useful knowledge about accommodation, handling and various trial procedures. The website also provides information about the recommended structure of the animal welfare body and information about the tasks that the animal welfare body must manage.

The committee's website is also where you will find information about the Annual Meeting of the Animal Welfare Bodies.



DENMARK'S 3R SYMPOSIUM 2022

Janne Koch (LEO Pharma), Merel Ritskes-Hoitinga (Aarhus University/Universiteit Utrecht), Jennifer Rosowski (Charité), Jan Lund Ottesen (Novo Nordisk) and Stefan Hippensteil (Charité) made up a panel for a debate on thoughts about a future without laboratory animals from the perspectives of academia and the pharmaceutical industry (the three first-mentioned panellists are pictured here).







THE BOARD STEPS DOWN

The board steps down

The end of March 2023 marks the expiry of the term of the current board, and a new board will see the light of day. The six original members share some of their thoughts how the Danish 3R-Center has developed since it was established ten years ago.

PHOTO: KIM GRANLI



Axel Kornerup Hansen

When we started the 3R-Center in 2013, my vision was to ensure real improvements in the welfare of animals used for research and development and to optimize the scientific results achieved from the individual animal, thus reducing the number of animals needed in each experiment. I still think it is a major problem that so many animals actually go to waste in research because animal experimentation often fails to yield results that are translatable to humans. I have embraced a vision that there will be a future in which we do not – merely for the sake of research – expose animals to stress beyond what we would accept for other animals under animal welfare legislation. We are still far from realizing this, which is why I still believe that more needs to be done to improve our use of animals, with better animal models and research methods that enable us to use fewer animals in relation to obtaining our health goals for humans.

I think that in the past 10 years, the 3R-Center has contributed substantially to approaching these objectives, but our resources are too limited to do the work at the 3R-Center that would enable Denmark to make significant progress within a short time horizon. 40



When the Danish 3R-Center started, I focused particularly on *Refinement* and advising scientists wanting to conduct animal experiments. This was mainly based on assessing the severity of the projects and focusing on methods to reduce the severity. My vision was to secure embedment of the 3R principles in research environments and society, which would be implemented through various dissemination efforts in both research environments and society at large – such as at the annual science festival for school students, Science Calling (*Videnskaben på Besøg*), where I have made a great number of talks about laboratory animals and the 3Rs. I definitely believe that the Danish 3R-Center has been able to communicate its message broadly.

Over the 10 years that have passed since the establishment of the 3R-Center, I have noticed a gradual shift in the board from an approach based on 3R principles alone to intensified focus on NAMs (Non-Animal Methods). I certainly approve of this change, which is why I still believe that going forward, the center should also include this wider area of focus.



In 2013, my vision for the 3R-Center involved that everyone should adhere to the 3R principles 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. I also wanted to set annual goals and take a strategic approach to the 3Rs to help ensure measurable results for the center (with a focus on the initiatives that are most likely to benefit laboratory animals in Denmark). I specifically wanted the 3R-Center to focus on 1) enhanced cooperation in the field of 3R; 2) improved communication of the 3R area; 3) increased 3R research in areas that can be put to immediate use; and 4) documentable results (in the short and long term).

I still have that focus today. It is probably down to individual opinion whether you believe that we have succeeded in only using the number of animals necessary wherever possible, but whether we did what we could to use an adequate number of animal-free methods is a matter of preference. There is probably more that could be done, but I actually believe that the Danish 3R-Center's board has worked to achieve all these goals. The long list of activities that were launched over the past 10 years and a review of our annual targets show that we made a difference. I hope that a new board with renewed energy will be able to boost this further.



Lisbeth E. Knudsen

In my application to become a board member in 2013, I wrote: "It is my vision to promote developments away from routine use of animals for testing by promoting the development of animal-free methods in safety testing as has already been the case for acute and local tolerance toxicology. It is also my vision to work for optimal conditions for laboratory animals with respectful and gentle treatment by promoting and raising awareness of current Danish initiatives in the industry and at universities and contributing to transparency in the use of laboratory animals. It is my clear impression that EU-funded biomedical research is moving towards increased use of voluntary subjects, including children, after thorough advance approval of protocols and the necessary ethical and data confidentiality conditions that safeguard participants. Animal experimentation should only be used for mechanistic whole-body studies that cannot be conducted in humans for ethical reasons and only when already available data, in vitro methods, analogy conclusions and other replacement methods have been fully exhausted."

My visions remain the same and can be supplemented by acknowledging the increased interest in sharing data to avoid unnecessary repetition of experiments. The ten years on the board of the 3R-Center have presented challenges in relation to recognition of animal-free methods, which is the area that I have represented. As a positive step, the 3R-Center is now recognizing animal-free methods as eligible for support, also if they do not necessarily replace animal experimentation 1:1 but are considered part of an overall mechanism investigation.

Funding from the 3R-Center's very modest pool of DK 1.5 million for three Danish projects a year has contributed to promoting the animal-free approach.

Contacts with the regulators and academia outside Denmark have also helped align Denmark with visionary countries like Germany and Sweden.

The use of in vitro technologies and human-source material has also become more prevalent within the 3Rs where it is currently often the first-line choice to use cell cultures from humans and use tissue for models. Examples include using newborn placenta for experimental studies of transport of substances from mother to embryo.

The 3R-Center could benefit from spending the years ahead on consolidating animal-free methods by setting up core facilities and sharing more of the lessons learned in Denmark and abroad. I also consider it essential for the 3R-Center to continue educational activities at all levels and keep an eye on developments that depart from the routine use of laboratory animals for research and teaching in Denmark.



Christine Nellemann

In 2013, my 3R vision was 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.

Now, a decade later, we have come quite some way in achieving this. We have actually come so far that the 3R-Center has now proposed a vision that someday we can manage without using laboratory animals. This is no longer utopia. What will get us there is largely an integrated approach to the use of various models, but also tissue and data from humans. I am convinced that the 3R-Center will continue contributing to this.

One of my goals for the 3R-Center was that we should initiate new research in the field. We have succeeded in this, but also in securing broader awareness of the research and convincing several foundations to aim for their supported research to promote the 3Rs. This is the right direction.

I am proud of what we have achieved as the board of the 3R-Center in our first ten years, and I hope that the next ten will be equally successful.



Erwin Roggen

Erwin Roggen accompanied by Birgitte Kousholt in 2018 when Birgitte was awarded the annual 3R Prize. Birgitte Kousholt has subsequently become a member of the board of the Danish 3R-Center.

My 3R vision has always been to drive the application and acceptance (in industry and authorities alike) of testing methods and strategies that help safeguard health and safety without using animals. The increasing quality of applications for 3R research funding over the years, including animal-free methods, suggests that the Danish 3R-Center has made a positive difference.

The 3R-Center has also been successful in driving knowledge gathering and sharing, sharing experiences and establishing best 3R practices, communications, teaching/training and facilitation. However, there is still a long way before these methods become common and the first-line choice.

As targets for the 3R-Center's future work, I consider it important to aim to establish a mindset in the laboratory-animal environment where exploring animal-free methods is the first choice when testing, developing and marketing new products and only when such methods do not provide the solution move on to considering animal models.

As most academic research groups, but also small biotech companies, might find it expensive and financially difficult to achieve the ambition of establishing animal-free development environments, I believe that the future board should give highest priority to establishing such environments.

Vision for the **3R-Center** of the future

Almost ten years have passed since the center was established in 2013 and the board of the Danish 3R-Center defined a vision for guiding the work. Much has changed over the past decade, in terms of both laboratory animals and available alternatives, and the current board has consequently defined a vision that could inspire the future efforts of the Danish 3R-Center's board.

Vision

Tomorrow's Denmark does not involve the use of laboratory animals

Mission

The Danish 3R-Center will work to facilitate access to animal-free methods so that they eventually become as accessible as animal models:

- The Danish 3R-Center will seek to influence decision-makers to obtain more financial resources for developing animal-free methods.
- The Danish 3R-Center will spread the message about existing international facilities focused on the development and availability of animal-free methods.
- The Danish 3R-Center will work to establish core facilities in Denmark that can help resolve research questions without the use of animal models.¹

The Danish 3R-Center will work to enhance the impact of the 3Rs on Denmark's use of laboratory animals for the benefit of both laboratory animals and research results:

- The Danish 3R-Center will provide financial support for research projects that can reduce the use or strain on laboratory animals through *Replacement, Reduction* or *Refinement.*
- The Danish 3R-Center will increase knowledge about the 3Rs and progress being made in the field of 3Rs among everyone with a professional interest in laboratory animals and the general public.
- The Danish 3R-Center will work to establish and strengthen relations with international 3R organizations and other relevant stakeholders to benefit mutually from sharing knowledge of the 3Rs.

1: An example of this is Der Simulierte Mensch (Si-M) at Charité – Universitätsmedizin Berlin. Si-M is a building in which scientists will cooperate on simulating human cells and tissue by using new technologies such as 3D cultivation, multi-organ chips and 3D bioprinting that may promote an animal-free culture. The construction is expected to be ready in 2023.

Evaluation of the activities of the Danish 3R-Center 2013-2022

In 2021, the Danish 3R-Center decided to commission an evaluation report. At the time, the board had worked throughout two board terms of four years each, and there was a desire for an external assessment of the effort to learn whether the many years of work had been fruitful. The evaluation could also give a coming board a number of recommendations for their future work. The board decided to give this task to Aage Kristian Olsen Alstrup of Aarhus University based on his great insight into both laboratory animals and the 3Rs. The following is a summary of the evaluation. The full text is available on the 3R-Center's website.

Evaluation of the activities of the Danish 3R-Center 2013-2022

Summary

This evaluation concerns the activities of the Danish 3R-Center and the National Committee from their establishment in 2013 until today (2022). The evaluation focuses primarily on the distribution of 3R research funding, organizing the annual 3R symposium and the annual meeting of the animal welfare bodies as well as communication about animal experimentation and the 3Rs in Denmark. The evaluation builds on available material provided by the 3R-Center as well as interviews with grant holders, participants at the annual symposium and annual meeting as well as lower secondary school and youth education teachers.

Overall, the evaluation shows that the sparse research funding distributed has yielded comparatively good results, though several researchers ask for larger grants. A satisfactory number of articles have been published, and they are well-cited in international literature. The 3R symposiums and annual meetings have been well-attended with highly satisfied participants. Dialogue is a key element in both the symposiums and the annual meetings and is at least as important as the many presentations given. The teaching materials for lower and upper secondary schools are well-suited for the target groups, but the material is less well known among teachers at Danish schools, unfortunately. On the other hand, the Danish 3R-Center's website is frequently visited by those interested in learning more about the 3Rs. This is particularly true of the Danish pages. The overall conclusion is that the Danish 3R-Center and the National Committee have had a considerable positive impact on 3Rs in Denmark. This will both benefit laboratory animals and ensure a good dialogue between those working with laboratory animals and animal welfare.





DENMARK'S **3R SYMPOSIUM** 2022

Stephan Rosshart, University Medical Center be Wild – Wildlings a novel translational

Thomas Bouquin, Sanofi, rounded off two good days with the presentation Strengths and weakness of non-animal derived therapeutic antibodies. \checkmark

Freiburg, gave the presentation Born to research model for human diseases. \uparrow elle





APPENDIX

Appendix

The following is an overview of all the projects that have received support since the Danish 3R-Center was founded in 2013.

Overview of supported projects, 2014–2021

Project	R	Project manager	Status	Publication
2014				
'Artificial blood vessels' – a model for investigating diabetic arteri- osclerosis	Replacement	Mette Bjerre Aarhus University	Completed	To be published once additi- onal studies have been done and more results achieved
Standardizing gut microbiota in mice as a tool for reducing the number of animals in the indivi- dual experiments	Reduction	Axel Kornerup Hansen University of Copenhagen	Completed	Published in Scientific Reports in March 2017 (link at the Danish 3R-Center's website)
Refinement of animal models of pain: Development of methods to limit pain in laboratory rats used in pain research	Refinement	Klas Abelson University of Copenhagen	Completed	Published in the Scandinavian Journal of Laboratory Animal Science, March 2020. (link at the Danish 3R-Center's website) Plos One in January 2020 (link at the Danish 3R-Center's website)
Pathological and immunological consequences of murine blood sampling	Refinement	Dorte Bratbo Sørensen University of Copenhagen	Completed	Published in the Journal of the American Association for Laboratory Animal Science in May 2019 (link at the Danish 3R-Center's website)
2015				
Developing an in vitro method to predict acute pulmonary toxicity from aerosol proofing products	Replacement	Jorid Birkelund Sørli (previously Søren Thor Larsen) The National Research Centre for the Working Environment	Completed	Published in ALTEX Online First in August 2017 (link at the Danish 3R-Center's website)
Can chickens be immunized with an aerosol combined with vacci- nation? Investigating a non-in- vasive method for production of antibodies	Refinement	Otto Kalliokoski University of Copenhagen	Completed	Not published (negative results) (Link to the report on the Danish 3R-Center's website)
Artificial skin in a Petri dish as an alternative to laboratory animals	Replacement	Mette Elena Skindersø (June Lissa Hansen) Statens Serum Institut	Completed	Publication expected in the near future (link on the Danish 3R-Center's website)

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Project	R	Project manager	Status	Publication
2016				
Development of computer models for predicting chemicals' impact on thyroid hormones	Replacement	Marianne Dybdahl DTU FOOD	Completed	Published in Computational Toxicology in January 2017 and Computational Toxicology in 2017 (link at the Danish 3R-Center's website)
Using cell cultures to minimize the need for laboratory animals in developing and manufacturing vaccines for farmed fish	Reduction/ Refinement	Niels Lorenzen University of Aarhus	Completed	Supplementary experiments must be conducted to enable publication
Towards better treatment of brain cancer with new cell-based models and less animal testing	Replacement	Bjarne Winther Kristensen University of Southern Denmark	Completed	Published in the Journal of Neu- ro-Oncology in August 2016 and PLOS ONE in May 2016 (link at the 3R-Center's website)
2017				
The use of primary kidney isola- tes from humans for studying the molecular aspects of blood-pres- sure regulation	Replacement	Henrik Dimke University of Southern Denmark	Completed	No plans for publication
Implementation of pain-treat- ment methods for rats used as a model for inflammatory arthritic pain	Refinement	Klas Abelson University of Copenhagen	Completed	Publication expected in the near future
Development of cell-based assays for measuring antibo- dy-mediated protection against the chlamydia bacterium	Replacement	Jes Dietrich Statens Serum Institut	Completed	Published in the Journal of Quantitative Cell Sciences in March 2018 (link at the Danish 3R-Center's website)
2018				
Murine Passport	Reduction	Axel Kornerup Hansen University of Copenhagen	Completed	Published in Scientific Reports in August 2022 (link at the Danish 3R-Center's website)
New advanced blood-infection model	Replacement	Thomas Emil Andersen University of Southern Denmark	Completed	Published in Scientific Reports in March 2021 (link at the Danish 3R-Center's website)
Transport and metabolism of fun- gicides in the human placenta	Replacement	Bjarne Styrishave University of Copenhagen	Completed	Publication expected (link on the Danish 3R-Center's website)
2019				
The significance of cage enrichment for protein metabolic experiments	Refinement	Helle Nygaard Lærke Aarhus University	Completed	Publication expected
Genetic murine modification without a need for extensive breeding	Reduction	Per Svenningsen University of Southern Denmark	Completed	No plans for publication
Study and analysis of internal validity with Danish preclinical research	Reduction	Birgitte S. Kousholt Aarhus University	Completed	Published in PLOS ONE in November 2022 (link at the Danish 3R-Center's website)

Project	R	Project manager	Status	Publication
2020 New methods for exploring the interplay of cells, surrounding tissue and mechanical forces in vascular disorders	Replacement	Julián Albarrán-Juárez Aarhus University	Completed	Published in Cells in August 2021 (link at the Danish 3R-Center's website).
Meta-analyses to identify the shortcomings of behavioural testing in preclinical medicine	Replacement	Otto Kalliokoski University of Copenhagen	Expected to be completed in May 2023	
Introduction of new human <i>ex</i> vivo model systems to study tumorigenesis in kidney cancer	Replacement	Kirsten Madsen University of Southern Denmark	Expected to be completed in 2023	
2021 Human derived blood-brain- barrier spheroids to study brain infections	Replacement	Yvonne Adams University of Copenhagen	Expected to be completed in 2023	
Automated monitoring in animal models for studies of vaccines against viral infections	Reduction Refinement	Gabriel Pedersen Statens Serum Institut	Expected to be completed in 2023	
Cellular heterogeneity as pre- dictor for the differentiation and regenerative potential of bone marrow stromal cells	Replacement	Ali Jasim Mohammad Jamil University of Southern Denmark	Expected to be completed in March 2024	
2022 Use of ultrasound for early diag- nosis of necrotizing enterocolitis in laboratory premature piglets	Refinement	Thomas Thymann University of Copenhagen	Expected to be completed in September 2023	
PluriLum assay: A novel stem cell-based assay for testing of chemicals' embryotoxic effects	Replacement	Rie Vinggaard Technical University of Denmark	Expected to be completed in September 2023	
Replacement of meningioma ani- mal models with a meningioma ex vivo/organoid model to test pharmacological advances in meningioma treatment	Replacement	Mikkel Schou Andersen University of Southern Denmark	Expected to be completed in September 2023	

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Statements, recommendations and reports

The Danish 3R-Center's board and the National Committee for the Protection of Animals used for Scientific Purposes have published the following statements, recommendations and reports:

- The Danish 3R-Center thinks that a broader understanding of the concept of replacement of experiments on animals is needed (Danish 3R-Center's board, 2019).
- Danish animal welfare bodies function excellently (Statement by the National Committee for the Protection of Animals used for Scientific Purposes, 2021).
- A higher number of laboratory animals in Denmark is not necessarily negative (National Committee for the Protection of Animals used for Scientific Purposes/the Danish 3R-Center, 2018).
- Recommendation on antibodies (Statement by the National Committee for the Protection of Animals used for Scientific Purposes, 2021).

- Use of animals in teaching (Statement by the Danish 3R-Center and the National Committee for the Protection of Animals used for Scientific Purposes, 2021).
- Laboratory-animal facilities and COVID-19 (Report prepared by the National Committee for the Protection of Animals used for Scientific Purposes/the Danish 3R-Center, March 2022)
- Experimental animals with the highest degree of suffering in 2021 (Report prepared by the Danish 3R-Center, March 2022)

READ MORE ON EN.3RCENTER.DK

These are all available (in Danish) on the Danish 3R-Center's website: **3rcenter.dk/om-3r-centeret/udtalelser**

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DK-2600 Glostrup, Denmark +45 7227 6900 info@3rcenter.dk www.en.3rcenter.dk ISBN no.: 978-87-93147-52-2 Photo: The Danish 3R-Center, Kim Granli, Unsplash Design: Essensen

READ MORE ON EN.3RCENTER.DK

The Danish 3R-Center in brief

The Danish 3R-Center is a collaboration involving the Ministry of Food, Agriculture and Fishery, the Danish Animal Welfare Society, DOSO, LEO Pharma, Lundbeck and Novo Nordisk.The Danish 3R-Center works to promote the 3Rs in Denmark to bring focus to bear on alternatives to animal experimentation and create even better conditions for laboratory animals.

The Danish Animal Welfare Society

"In the view of the Danish Animal Welfare Society, the use of laboratory animals must be restricted wherever possible. This is why the Danish Animal Welfare Society actively supports the Danish 3R-Center's efforts to replace, reduce and refine the use of laboratory animals. In particular, we have great confidence that efforts to promote the development and knowledge of alternatives to laboratory animals will contribute to realizing DAWS' ambition to phase out the use of laboratory animals."

DOSO (Cooperation Organisation of Animal Welfare Bodies)

"The overarching aim of DOSO is to abolish animal experimentation. Towards this end, DOSO is actively engaged in promoting the development, validation and implementation of alternatives to animal testing based on the 3R principles. Making an active effort and supporting the Danish 3R-Center provides a good opportunity to achieve this goal."

LEO Pharma

"Many of our tests and testing models in the development of pharmaceuticals for treating skin disorders have been replaced in part by laboratory testing. However, we still need to use laboratory animals in the development of medicine to reliably assess the efficacy of medicines and comply with regulatory requirements. At LEO Pharma, our animal welfare policy revolves around on the 3Rs, and we have ongoing initiatives to reduce, replace and refine the use of laboratory animals wherever 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. We continuously seek to optimize the conditions for these animals, and we use alternative methods wherever 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 still not possible to develop medicine that is efficacious and safe for patients without using laboratory animals. Novo Nordisk and the Danish 3R-Center share the same wish to promote the development of alternatives to animal testing, reduce the use of laboratory animals, improve conditions for laboratory animals and disseminate knowledge about alternatives to animal testing. Novo Nordisk actively works to achieve these goals, which is why we also actively support the Danish 3R-Center."