

## Table of content

Table of content	1
1. Focus on: Training of animals with emphasis of culture of care	4
A more natural working environment for rats - and animal caretakers	5
Refinement of the obese minipig model.	6
Socialization of pigs and rabbits - preparation for training	7
2. Focus on: Running a zebrafish facility	8
3. Focus on: Culture of care and 3Rs in practice – how to make a change	9
Correlation of amount of health notifications and genetical status, temperature and humidity in two rodent facilities.	10
Culture of Care – what does it look like at Novo Nordisk	11
Environmental enrichment as a factor in improving the welfare of laboratory mice	12
Implementing cup and tunnel handling in a pharmaceutical rodent facility	13
Insight - Practical implementation of laboratory animal monitoring and veterinary care	14
It is time we tell our stories. A call for engagement in science communication.	15
Learning log as a tool for culture of care	16
Mice can also be in Zen	17
To what extent is ‘culture of care’ and application of the 3Rs visible in Swedish applications for ethical review of animal research?	18
4. Focus on: Novel approaches to 3Rs in Practice	19
A Swedish strategy to replace animal experimentation	20
Automated Blood Sampling; does it contribute to the 3R's?	21
Improvement of inoculation method of the syngeneic mouse tumor model, MC38, within the principles of the 3Rs, to reduce tumor ulceration prevalence	22
Mouse MApp: an AI based app for animal welfare scoring	23
Ways to avoid harmful phenotype – implementation of dTag degron system	24
5. Focus on: Culture of care in research on wild animals	25
6. Focus on: The 3Rs in education	26
Animal Research:Time to Talk!	27
Animal technicians: The key persons for practical skills and competences	28
Assuring competence in practical tasks- ETPLAS working group 4	29
Models and simulators that can replace use of live animals in education	30
7. Focus on: Pain management	31
Acute phase proteins as inflammatory markers of Respiratory Disease (RD) in dairy calves	32
Effects of analgesia on functional outcome and well-being of rats subjected to spinal cord injury	33
8. Focus on: Male mouse aggression	34
Aggression in group-housed male mice: a dual approach to increased understanding	35
Housing male mice with ovariectomized females can overcome limitations of male mice group housing in long term studies	36
Illustrating behavioral epidemiology as a 3Rs solution to complex multifactorial problems: Fighting in mice as a worked example	37
9. Focus on: Monitoring animals under anesthesia	38

Monitoring animals under anesthesia in the wild	39
10. Focus on: Refinement	40
Discovering stress-free biomarkers in various mouse models using digital ventilated cages (DVC®) technology	41
Evaluation of 4 different nesting materials for laboratory mice	42
Norecopa: PREPARE for Better Science	43
Refined management and care of newborn Göttingen minipigs	44
Swedish acclimatization guidelines for rats and mice	45
The mouse in the house - an alternative to tail handling	46
We do not need to, and should not, fast our animals before the sucrose preference test for depression	47
11. Focus on: Reduction	48
Please stop using and teaching the Resource Equation	49
12. Focus on: Culture of care at the vendors	50
Dry Heat Sterilization in Vivariums – An Alternative Option for Sterilization in the Lab Animal Facility	51
Implementing change- creating a stimulating and cooperative environment for Göttingen Minipigs	52
Invited speakers	53
A standardised score sheet template for assessing rodent health	54
Assessing animal welfare – the familiar and the ‘exotic’	55
Behavioral profiling of rats, mice and zebrafish	56
BEST ANAESTHESIA PROTOCOL FOR FISH SPECIES AND SITUATION	57
Cage change on demand, challenges with implementation	58
Clinical training center	59
Culture of Care and 3Rs in practice- How to make people and organisations tick	60
Culture of care in the wild - does anyone care?	61
Culture of Care – What do we mean and how do we achieve the benefits?	62
Designing and running a zebrafish facility	63
Designing your own cardboard mouse house	64
Effective pain management in laboratory animals	65
Establishment of a searchable database for sharing tissues from experimental animals	66
Focus on novel approaches: Home cage monitoring of mice	67
Learning from failure- fostering the culture of care	68
Modelling the child eye cancer retinoblastoma using chicken embryonic retina	69
Monitoring long-term anesthesia in pigs	70
Monitoring pain in zebrafish	71
Non-aversive mouse handling at Lund University and the use of cage ladders	72
Optimizing zebrafish rearing – effects of environmental enrichment and fish density	73
Plan your breeding to avoid producing an excess of animals	74
Rabbit Human Habituation Program During Breeding Reduced Significantly Stress Related Signs During Acclimatization Period	75
Refined methods for picking up rodents – handling change in practice	76
Rodent anesthesia: optimization, refinement, and monitoring	77

STAY OUT OF MY TERRITORY - understanding and mitigating male mouse aggression	78
Swedish presidency; What happens in the EU related to the animal welfare legislation?	79
the legal use of biocides within a biomedical facility under the EU biocidal products regulations (BPR)	80
Their care is in our hands	81
Training of pigs in experimental studies	82
Use of scoring sheets for determination of human endpoints	83
Working with non-human primates: a challenge that requires daily commitment, patience and resourcefulness.	84
Workshop on choosing the right experimental design	85

# **1. Focus on: Training of animals with emphasis of culture of care**

# A more natural working environment for rats - and animal caretakers

1. Focus on: Training of animals with emphasis of culture of care

**Camilla Falk Bulow Clausen**<sup>1</sup>, *Maria Kiersgaard*<sup>1</sup>, *Helle Nordahl Hansen*<sup>1</sup>

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## **Introduction**

We wanted to work in a more cooperative way with our rats, and house them in a manner that are more natural. However we had a lot of challenges with clicker training, with practicalities as well as with the need of being able to execute the studies flawlessly.

## **Methods**

Here we present our new combination of housing and training rats. In this new set-up, we accommodate the natural group living environment of rats, different study requirements, as well as more satisfying work with animals, instead of heavy lifting of material.

In the wild, rats live in large family groups, each having their own defined place in the group, some being the more explorative types, others less so. In our housing environment we can accommodate both the larger group as well as a study related need for small group housing of only two rats together, and even single housing is possible.

## **Results**

We use CAT training (Constructional Approach Treatment) to socialize and habituate our rats to humans, and after a while they are confident and curious around us. This training allows us to cooperate with the animals.

## **Conclusion**

The method we are presenting is beneficial to both the rats, the study and the animal caretakers. The rats have a more natural housing, with exploration of "new territory" regularly, the study requirement of food intake measurements is honoured and the animal caretakers work more with animals and less with equipment.

## **Refinement of the obese minipig model.**

1. Focus on: Training of animals with emphasis of culture of care

**Cathrine Juel Bundgaard**<sup>1, 2</sup>

<sup>1</sup> drenth Larsen, Stine

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### **Introduction**

When exploring obesity-related diseases and seeking medication for treatment of obesity, we often use minipigs fed a high-fat diet ad libitum, resulting in severe obesity. This results in animals that will be in risk of having joint problems, such as osteoarthritis and musculoskeletal discomfort and psychological problems as depression. This is further problematic, since the obese minipigs are significantly less active than non-obese minipigs, particularly when there is free choice of activity or no activity. This has in previous studies led to problems with recurrent lameness and depressed (angry) animals

### **Methods**

In this study we had 24 minipigs weighing between 57-115 kg with a body fat percentage of around 40%. The study lasted for 7 months. To motivate the animals to move around, we let the animals get out of their pen to a playing area or an outside playing area every day, together with other animals in playgroups. We ensured that there were different toys in the play area to motivate the animals to move around. Furthermore we trained the animals to go for fitness walks on a leash, with an animal caretaker taking them for walks outside the stable. We started the training before the animal was obese, making it a daily routine.

### **Results**

The study lasted for 7 months and we had no animals experiencing lameness. Only one minipig seemed depressed.

### **Conclusion**

Motivating obese minipigs to move around and ensuring a mild exercise schedule, completely prevented the occurrence of lameness and depression we normally experience in this animal model.

# Socialization of pigs and rabbits - preparation for training

1. Focus on: Training of animals with emphasis of culture of care

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## Introduction

As animal care takers, we want to work with the animals in the best possible way, for both care taker and animal. Over the last couple of years, the number of in-house studies in pigs have increased, and we saw it necessary to improve our approach to handling and socialization. In our rabbit facility we also wanted to improve our approach to handling.

At Novo Nordisk we have refined our interaction with the animals during the acclimation period by incorporating socialization, habituation and training. As the animals are participating in studies by a varying time frame, the training is scalable and study dependent. This has resulted in improved animal welfare, while creating a better work environment for technical and scientific staff.

## Methods

I want to share our approach to socialization, habituation of pigs, and how we implemented this, and what the long-term benefits will be.

During the acclimation period we exercise the pigs with different enrichments, outdoor facilities, toys, food and hands-on handling.

## Results

I will also touch upon socialization and enrichment initiatives in our rabbit facility, e.g. marking, housing and handling.

I will also give examples of standard training and more complex study specific training and demonstrate this with pictures and videos.

## Conclusion

At Novo Nordisk we socialize our animals to improve the welfare, with this approach comes reduced stressed, potentially more reliable data and more accurate dosing, easier cooperation, and calmer animals.

## **2. Focus on: Running a zebrafish facility**



# **3. Focus on: Culture of care and 3Rs in practice - how to make a change**

## **Correlation of amount of health notifications and genetical status, temperature and humidity in two rodent facilities.**

3. Focus on: Culture of care and 3Rs in practice - how to make a change

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### **Introduction**

Recording clinical symptoms and developing systematic methods to evaluate animals' wellbeing may offer possibilities to detect earlier humane end points (HEP), help in assigning the actual severity class and retrospective assessment of project licenses as well as improve welfare during maintenance and experimental practices.

### **Methods**

In addition to genetic and microbiological status, environmental factors in laboratory animal facilities such as temperature (T) and relative humidity (RH) are important for the health of laboratory animals. We retrospectively collected data regarding amount and category of health notifications and correlated the number of notifications with RH and T and genetic status of laboratory animals as denoted GS1 (non-genetically modified = non-GM), GS2 (GM without harmful phenotype) and GS3 (GM with harmful phenotype) from two rodent facilities from 2019 and 2020.

### **Results**

Skin symptoms (wounds/scratches/bites) was the most common symptom category (approx. 35%) followed by loss of body weight/dehydration, fur condition/appearances, eyes, posture, delivery problems and moving/tremors (each approx. 10 %). When only the symptoms of GS3 category animals were evaluated, skin symptoms remained the most common category (approx. 30%), however, abnormal posture, loss of body weight, eyes, moving/tremors were more common symptom categories in GS3 category animals. Statistically significant correlation was detected between amount of health notifications and genetical status but not with the animal room T or RH.

### **Conclusion**

Our data indicate that genetically altered animals and those with naturally occurring mutations have higher propensity for developing health problems and their phenotype should be often evaluated to improve their health and welfare.

## **Culture of Care - what does it look like at Novo Nordisk**

3. Focus on: Culture of care and 3Rs in practice - how to make a change

**Cathrine Juel Bundgaard**<sup>1</sup>

<sup>1</sup> mikkelsen, johan

### **Introduction**

The concept of Culture of Care has gained much attention in recent years after it was introduced in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Many user establishments working with laboratory animals have worked with implementing or developing their own and unique culture when working with animal welfare and the 3 Rs.

### **Methods**

At Novo Nordisk our approach to Culture of Care is what we proactively choose to do when we put legislative requirements into action. We take a close look into the intentions of the legal requirements and from that, we choose the solutions which has the best match in accordance with the intentions. In other words, - we go above and beyond the minimum requirements of legislation. Culture of Care also includes the staff working with the animals, as they are the ones providing care to and show empathy with the animals.

### **Results**

The purpose of working with Culture of Care is to better enable us to reach our goals in terms of optimising the welfare of the animals and of the people working with them.

### **Conclusion**

The presentation will go through examples of Culture of Care in four phases of a study: Planning, Acclimatization, Study phase and End of Study. We will also explain how our approach supports establishing relationships and collects relevant input from all staff functions. It will also describe our mind-set as well as the supportive elements needed for a healthy Culture of Care.

## **Environmental enrichment as a factor in improving the welfare of laboratory mice**

3. Focus on: Culture of care and 3Rs in practice – how to make a change

**Katarzyna Dębek<sup>1</sup>**, *Karolina Jaworska<sup>2</sup>*, *Paulina Kałabun<sup>1</sup>*, *Monika Kwiatkowska<sup>1</sup>*

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### **Introduction**

Enrichment is any change we make to a given animal environment that is designed to aimed at cognitive, sensory, physical and social stimulation. The most commonly used enrichment in research work with rodents are shelters in the form of houses and tunnels, nesting material and reels.

### **Methods**

Our goal was to determine whether preference for the type of enrichment depends on the strain or group of animals. We tested 3 strains of laboratory mice. From each strain, both females and males. The age of the mice varied between 2 and 5 months.

### **Results**

The response time of mice to environmental enrichment differed between strains. The differences were up to several minutes. Often the age and sex of the animals affected the choice of environmental enrichments. F1 mice (B6xCBA) showed interest in paper tubes after a time of 3 minutes. Young mice were more interested in environmental enrichments.

### **Conclusion**

Environmental enrichments have a positive effect on maintaining high animal welfare laboratory animals and improve breeding parameters. They also activate the behavior of animals and increase their diversity. Animals can make activity choices and take control over the use of a given environment. It has also been shown that the use of enrichment minimizes the incidence of undesirable behaviors such as aggression or stereotypies. Another important aspect is the maintenance of species-typical behavior (e.g., nest building, chewing)

# Implementing cup and tunnel handling in a pharmaceutical rodent facility

3. Focus on: Culture of care and 3Rs in practice - how to make a change

**Maria Kiersgaard<sup>1</sup>**, *Marie Petersen<sup>1</sup>*, *Peter Lund Gade<sup>1</sup>*, *Helle Nordahl Hansen<sup>1</sup>*

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## Introduction

The way of handling mice has been by catching the animal by the tail, lifting it and generally holding the mouse by the tail always, this was considered the only effective and safe way of handling.

In 2010 the first article on cup and tunnel handling was published (Hurst et al., 2010). This and following articles demonstrated that catching, lifting and handling mice by either cup or tunnel handling is less stressful and creates more trust for the mice.

Hurst, J. and West, R. (2010). Taming anxiety in laboratory mice. *Nat. Methods* 7, 825-826 doi:10.1038/nmeth.1500

## Methods

In 2015, we began to look into cup and tunnel handling, however, without the correct instructions, it was unsuccessful.

In 2017 a decision was taken at management level, that cup and tunnel handling should be implemented in 2018.

Emphasis was on the following:

- Employees had to change their way of thinking, as well as their way of acting.
- The employees had to be educated in both the theory as well as the practical aspect of the new methods before beginning the implementation.
- Time had to be dedicated to training and evaluating.
- Equipment (tunnels) had to be in place.

With a dedicated plan and intensive follow-up, a full implementation has been reached, as well as a mind-set change amongst the caretakers.

## Results

Tunnel and cup handling of mice can be applied in any animal facility. However, training of personnel and management endorsement is essential, and if tunnel handling is desired, tunnels must be provided.

## Conclusion

Successfully implemented.

# Insight - Practical implementation of laboratory animal monitoring and veterinary care

3. Focus on: Culture of care and 3Rs in practice - how to make a change

**Søren Søgaard**<sup>1</sup>, *Marianne Ravn Møller*<sup>1</sup>

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## Introduction

Working with a variety of animal models in metabolic disease areas we wanted to implement a continuous loop of animal monitoring and rapid veterinary care to ensure the wellbeing of the animals and highlight a culture of care.

## Methods

Our animal care program now consists of a cooperation between study directors, animal technicians and laboratory animal veterinarians to ensure an efficient daily monitoring of the animals. The monitoring consists of both objective parameters (e.g., body weight or food intake), but also by clinical parameters consisting of daily visual inspections by the animal caretakers and veterinarians.

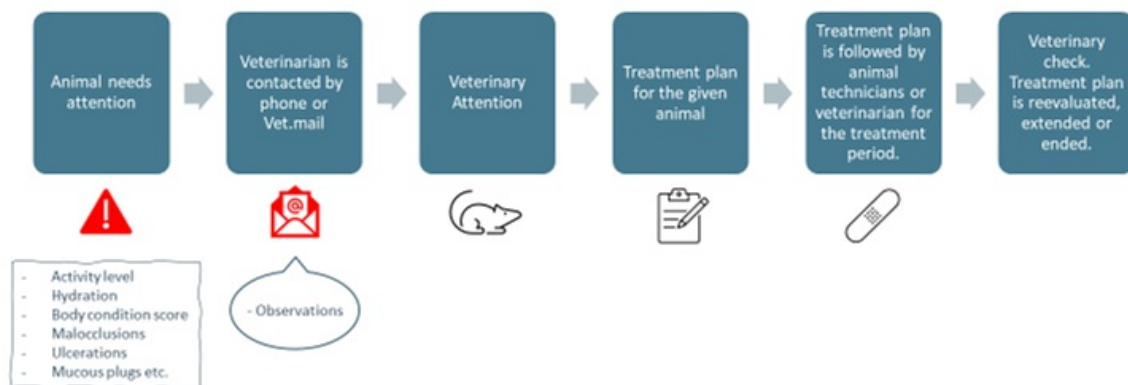
Together with our internal computational biology team, we have developed a weight monitoring system for the animals in which data is immediately uploaded directly to our internal data explorer. Giving both study directors and veterinarians an excellent tool for monitoring the animals and ensure that animals also without clinical or external signs of disease will be assessed daily based on differences on objective parameters also.

## Results

Animal caretakers are instructed to look for a variety of parameters when handling the animals. If veterinary attention is required, the observations are passed on through a designated email that are pushed to the attending veterinarians who will then attend the animal facility within minutes for evaluation and scheduling of a treatment plan. Animals in treatment are incorporated into an internal planning tool and becomes part of the daily schedule.

## Conclusion

These measures have led to a rapid response time while assuring that animals receive the proper treatment and care they need.



## **It is time we tell our stories. A call for engagement in science communication.**

3. Focus on: Culture of care and 3Rs in practice – how to make a change

**Jenny Berrio**<sup>1</sup>

<sup>1</sup> Department of Experimental Medicine. Section of Research and Education. Faculty of Health and Medical sciences. University of Copenhagen.

### **Introduction**

The contributions of animal experimentation to society are irrefutable. The need to continue it for the foreseeable future is also hard to challenge. Yet, public opinion on the matter is divided. Popular initiatives to stop animal experimentation have gone on the ballot and animal rights groups are getting louder while we, those in the field, are mostly silent. Unfortunately, a distorted version of the reality of animal experimentation is being broadcasted to the public by groups of people skilled at emotionally engaging society.

### **Methods**

As a PhD student, I thought there was little I could do to improve the situation, but I was wrong. Researchers at all levels and their supporting staff are indeed key assets in the quest to defend animal experimentation against public misperception.

### **Results**

I would like to share the lessons I took from the first workshop on effective science communication for early stage researchers by the League of European Research Universities (LERU).

### **Conclusion**

This is a call for a more active engagement in science communication. Let us tell our stories and share our reality.

## Learning log as a tool for culture of care

3. Focus on: Culture of care and 3Rs in practice – how to make a change

**Therese Edström**<sup>1</sup>

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### **Introduction**

Culture of CareA culture of care ensuring the humane care and use of animals in research is paramount. Such a culture starts with the people, so everyone is confident in their team, so mistakes lead to learning without risk of being punished and so helping each other for the good of everyone and for the best of the animals is the norm.

### **Methods**

Learning LogThe Learning Log is a tool for reporting events which have or could have an impact on animal welfare and study integrity. Since study integrity issues can lead to the risk of a study being repeated, this is potentially an animal welfare concern.

The learning log is accessible for staff involved in animal care and use, using a mobile phone QR code or a computer using links.

### **Results**

The event report is picked up by the Animal Welfare Officer, who investigates *what* has happened with a focus on the root causes of the event rather than *who* was guilty of wrongdoing. The investigation may involve a thorough Root Cause Analysis (RCA) or can be limited to interviews with involved persons to capture causative reasons in the categories Knowledge and Skills or Methods and/or Resources, whereafter Corrective and Preventative Actions (CAPAs) are suggested. Actions to stop any ongoing animal suffering must of course be done as soon as detected.

### **Conclusion**

Finally, the learnings and CAPAs are presented on Learnings Cards that are shared across the AstraZeneca *in vivo* community.



## Mice can also be in Zen

3. Focus on: Culture of care and 3Rs in practice – how to make a change

**Maria Kiersgaard<sup>1</sup>**, *Marie Petersen<sup>1</sup>*, *Helle Nordahl Hansen<sup>1</sup>*

<sup>1</sup> Novo Nordisk A/S

### **Introduction**

Bodyweight is an important output in experimental animal work, both as a scientific endpoint, as well as an animal welfare assessment.

### **Methods**

When weighing mice, we have previously used “weighing boxes” for the animals. The mice often seemed somewhat distressed in the box and tried to jump out, sometimes with success.

When switching handling method, from tail handling to cup and tunnel handling, we found it very difficult to use the weighing boxes. Since we observed that the mice seemed calmer with the new handling methods, we wondered if the weighing boxes were necessary anymore.

### **Results**

We now use yoga mats, cut to fit the scale. The mice keep calmly on the yoga mat, being weighed and voluntarily enters the tunnel or palm of hand, when weighing is over.

The yoga mats can be washed and wiped with ethanol when necessary, but the mice urinate a lot less on the mat, than they did in the weighing box, this provide food for thought.

### **Conclusion**

Everybody, both mice and animal caretakers, are very happy with this change, which is so easy and yet makes a huge difference.

## To what extent is ‘culture of care’ and application of the 3Rs visible in Swedish applications for ethical review of animal research?

3. Focus on: Culture of care and 3Rs in practice – how to make a change

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<sup>1</sup> Swedish University of Agricultural Sciences

### **Introduction**

*Directive 2010/63/EU* states that a “climate of care” should be cultivated within breeding, keeping and use of research animals and that “animal-welfare considerations should be given the highest priority”. It has been in place for more than a decade and a relevant question to ask is thus how well the member states are living up to this goal.

### **Methods**

Consideration, application and fulfilment of the 3Rs from project-planning to retrospective review is a vital part of maintaining a culture of care. To investigate how the 3Rs are described in Swedish animal ethics applications, our research group created a template based on content requirements derived from current regulations and used this to analyse 44 applications of ethical review.

### **Results**

Our results show that project proposals frequently don’t fulfil regulatory 3R criteria and that despite this, they are approved by the competent authorities. Furthermore, replacement, reduction and refinement are not seldom confused with, and described as, one another by applying researchers.

### **Conclusion**

Inadequate application of the 3Rs puts animal wellbeing at risk and insufficient or inaccurate description of them in applications makes the task of the competent authorities unnecessarily difficult. We suggest that to mitigate these issues, researchers’ knowledge of the 3Rs needs to be improved and more emphasis be put on the 3Rs in the application form template. Only if the 3Rs are sufficiently applied and thoroughly described in the applications can the animals’ situation be fully understood and the competent authorities ensure that only projects are approved where the benefits outweigh the harms.

# **4. Focus on: Novel approaches to 3Rs in Practice**

## A Swedish strategy to replace animal experimentation

4. Focus on: Novel approaches to 3Rs in Practice

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<sup>2</sup> Swedish National Committee for the Protection of Animals Used for Scientific Purposes, Swedish Board of Agriculture

<sup>3</sup> Swedish Fund for Research Without Animal Experiments

<sup>4</sup> University Veterinary, Faculty of Medicine, Lund University

### Introduction

The Swedish National Committee for the Protection of Animals Used for Scientific Purposes and its executive body the Swedish 3Rs Center have set out to compile a strategy to replace animal experimentation in a Swedish context.

### Methods

The project was kicked-off through an open workshop where stakeholders were invited to give input. Four different topics were discussed: positive attitudes towards development and implementation of replacement, how science communities can collaborate to drive the transition from animal use to alternative methods, areas where animal experimentation could be replaced in a near future, and finally, what obstacles are seen in the validation and evaluation process for new methods.

### Results

The discussions focused on funding, collaboration between researchers and the importance of visualizing information on non-animal methods. The information has been processed by the National Committee and the 3Rs Center and compiled into a working document.

Three overall focus areas have been postulated:

- Knowledge and collaboration on animal-free research shall increase
- Animal-free methods shall be developed and evaluated at a faster pace
- Animal-free methods shall be implemented and replace animal experimentation as soon as scientifically possible

### Conclusion

The project has now moved into the next phase – anchoring with the target groups. During this process, input will again be collected from stakeholders and the document modified to encompass their views. Ideas for specific interventions or activities will also be collected.

When finalized, the document will be used to direct the Swedish National Committee's work to replace animal experimentation and disseminated to other organizations as inspiration.

## **Automated Blood Sampling; does it contribute to the 3R's?**

4. Focus on: Novel approaches to 3Rs in Practice

**Edwin Spoelstra<sup>1</sup>**, *Xanne Ketelaar<sup>1</sup>*, *Horst Beier*

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### **Introduction**

Acute blood sampling techniques such as facial vein and tail vein are commonly used techniques to obtain blood samples from mice and rats. These acute techniques require the animal to be restrained; physically (immobilization) or chemically (anesthesia). Restraint compromises the "true" value of blood drug concentration. Freely moving blood sampling - indwelling catheters - is the preferred method to obtain these "true" values. Automated blood sampling (ABS) in combination with freely moving animals can further contribute for obtaining "true" values of blood drug concentration. ABS significantly contributes not only to the "true" blood drug value but moreover contributes to the reduction of animals needed in stress prone research. Park et al. were able to investigate the difference (significant) in stress response between wild type- and pendrin knock out mice.

### **Methods**

### **Results**

### **Conclusion**

# Improvement of inoculation method of the syngeneic mouse tumor model, MC38, within the principles of the 3Rs, to reduce tumor ulceration prevalence

4. Focus on: Novel approaches to 3Rs in Practice

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## Introduction

Syngeneic mouse tumor models are widely used as they retain an intact immune system, making them especially useful when investigating immunotherapies. However, a common problem is the development of tumor ulcerations which can have devastating consequences on the study outcome and increasing the number of animals used and causing distress of the animals. To overcome this issue, we evaluated the impact of different inoculation strategies on ulceration development in the syngeneic mouse tumor model MC38 with the aim of improving the experimental setup within the principles of the 3Rs.

## Methods

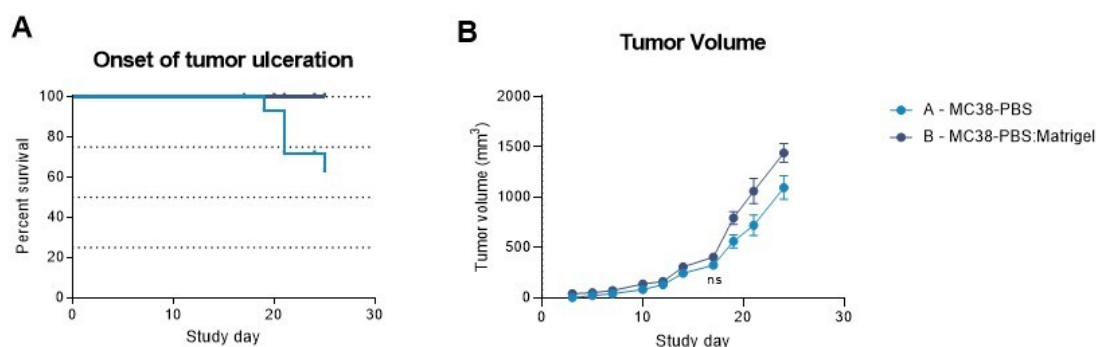
Mice were inoculated subcutaneously with  $0.3 \times 10^6$  MC38 cells resuspended in either PBS or PBS:Matrigel. Tumor and body weight measurements were performed three times every week and the appearance of tumor redness, sores or ulcerations were noted until humane endpoints had been reached. No dermal ointment was given if redness or sores on the tumor appeared.

## Results

Mice inoculated with MC38-PBS:Matrigel had significantly less redness and sores on the tumors compared to mice inoculated with MC38-PBS. Consequently, none of the mice inoculated with MC38-PBS:Matrigel had to be euthanized due to tumor ulcerations (Figure 1A). Both inoculation methods provided similar tumor growth ( $p=0.11$ , study day 17) (Figure 1B). Further investigations into this are currently ongoing.

## Conclusion

We have successfully reduced the frequency of tumor ulcerations in the syngeneic MC38-PBS:Matrigel mouse model from 33% (PBS) to 0% (PBS:Matrigel). This will decrease the number of animals that have to be used as well as the distress of the animals resulting in an improved study outcome.



## Mouse MApp: an AI based app for animal welfare scoring

4. Focus on: Novel approaches to 3Rs in Practice

**Lena William-Olsson<sup>1</sup>**, *Marie Johansson<sup>1</sup>, Frida Andersson<sup>1</sup>, Lynsey Frazer<sup>1</sup>, Elin Holmedal<sup>1</sup>*

<sup>1</sup> Animal Science and Technologies, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, Gothenburg, Sweden

### **Introduction**

There is a need for objective analysis of individual mouse wellbeing. By using artificial intelligence (AI) learning, human bias is taken out of the evaluation process. This allows each mouse to receive an accurate and consistent pain or body condition scoring for health and wellbeing tracking.

### **Methods**

The UK NC3Rs CRACK IT Challenge is a competition that funds collaborations between industry and academia to solve scientific and business challenges to improve animal welfare. AstraZeneca sponsors the Mouse MApp challenge and is working to support the winning team at Newcastle University to create a facial- and body recognition app based on AI and machine learning that automates detection of pain (Mouse Grimace Scale, MGS) and body condition (Body Condition Score, BCS).

### **Results**

Animal Science and technology scientists across sites at AstraZeneca have collected thousands of images, that together with manual BCS scores from mice of highly varying body composition form the base for the AI algorithms of the app development. Scientists at the consortium are currently in the process of launching a beta version of the app to be tested live.

### **Conclusion**

The CRACK IT challenges and this project in particular is a fundamental shift within the animal space to lean on and harness the benefits of AI technology. To develop an AI based app for animal welfare scoring is a scientific edge and a challenge that allows us to provide better animal care and better science while providing a better experience for our people-true culture of care.

## Ways to avoid harmful phenotype - implementation of dTag degraon system

4. Focus on: Novel approaches to 3Rs in Practice

**Katarzyna Sałas<sup>1</sup>**, *Marcin Szpila<sup>1, 2</sup>*, *Wiktor Antczak<sup>3</sup>*, *Andrzej Dziembowski<sup>3</sup>*, *Olga Gewartowska<sup>1</sup>*

<sup>1</sup> Genome Engineering Unit; International Institute of Molecular and Cell Biology in Warsaw

<sup>2</sup> Faculty of Biology; University of Warsaw

<sup>3</sup> Laboratory of RNA Biology; International Institute of Molecular and Cell Biology in Warsaw

### Introduction

CRISPR/Cas9 methodology has revolutionized generation of genetically modified organisms. For example, creating knock-out animals by random indels has astonishing efficiency. However, constitutive gene knock-outs often result in lethality or the development of noxious phenotypes.

### Methods

To overcome this issue, we have implemented the degraon (dTAG) system which allows for the depletion of proteins in a time-controlled manner. In this system, a small degrader tag (FKBP) is added to the endogenous protein but remains inactive until the addition of the ligand. Once the ligand is added, the fusion protein is ubiquitinated and degraded. The preserved wild-type phenotype of transgenic mice with implemented dTAG system pleads in favor of transgenic animal welfare improvement.

### Results

Within the last year, Genome Engineering Unit of IIMCB generated 8 different dTag mouse lines with an excellent result. Approximately 100 zygotes were injected to subject modification (mostly in only one round of microinjection), per one separate mouse line. Success rate of pups born with correct insertion was about 13%, and few had even homozygotic mutation. Here we present modification of gene encoding protein responsible for cytoplasmic polyadenylation. KO animals are characterized by low weight and hunched posture due to skeletal abnormalities, while mice tagged with FKBP tag looked completely normal.

### Conclusion

We show a very efficient method for the generation of knock-in animals with a degrader tag. Moreover, dTAG organisms can also provide a solution for research on essential genes, knockouts of which are embryo lethal. Implementing such models could open opportunities for novel research and improve laboratory animals' welfare.



# **5. Focus on: Culture of care in research on wild animals**

# **6. Focus on: The 3Rs in education**

# **Animal Research: Time to Talk!**

6. Focus on: The 3Rs in education

**Gege Lie**<sup>1</sup>

<sup>1</sup> European Animal Research Association

## **Introduction**

### **Animal Research: Time to Talk!**

In a number of European countries, public and private research institutions have made the bold decision to adopt new persuasive practices and policies to engage with the public on the use of animals in scientific and biomedical research. In Europe there are now eight National Transparency Agreements on animal research, Spain, Portugal, Belgium, France, Germany, Netherlands, Switzerland and the UK involving over 400 institutions. Institutions have collectively agreed to commitments on pursuing greater openness with the public. These commitments are that institutions; will be proactive in seeking opportunities to explain when, how and why they use animals in research; will provide information to the media and the general public about the conditions under which research using animals is carried out; will develop initiatives that generate greater public knowledge and understanding about the use of animals in scientific research; will place an animal welfare statement on their institution's website. The belief is that being more open and transparent about where they can and cannot be used, and where alternatives exist, will help improve public understanding. There is growing political pressure in Europe to transition towards 'animal free science'. The research community needs to improve communications about animals in research. This presentation will evaluate the experience in these countries, and explain why we need to talk about animal research.

#### **Methods**

Review of existing transparency agreements

#### **Results**

What has been achieved so far across eight countries

#### **Conclusion**

Lessons for others to learn

## **Animal technicians: The key persons for practical skills and competences**

6. Focus on: The 3Rs in education

Klas Abelson<sup>1</sup>, Åsa Holmberg<sup>1</sup>, Siri Knudsen<sup>1</sup>, **Lene Gorm Pedersen<sup>1</sup>**, Toomas Tiirats<sup>1</sup>, Hanna-Marja Voipio<sup>1</sup>

<sup>1</sup> Scand-LAS Education and Training Committee

### **Introduction**

Animal caretakers and technicians are the key persons for maintaining the highest possible level of technical skills and competences in an animal facility. This relates both to the technical work performed by the technical staff in their daily work, and to the role technicians may have in educating and training scientific staff involved in practical work at all levels.

### **Methods**

The Scand-LAS Committee for Education and Training acknowledges this important role of the technical staff, and strongly endorses activities to maintain and improve the skills and competences of this key category of staff. The committee has therefore initiated a visiting programme grant, where technicians can visit other workplaces and share and gain new knowledge among and between animal facilities in the Scand-LAS countries. The outline of this programme, as well as experience from a pilot study visit, will be presented in this session.

### **Results**

Furthermore, to emphasize the importance of the technicians' role in education and training of other staff, the session will present the organization of and experiences from CAREiN – a programme established in Norway on how to train the trainers.

### **Conclusion**

The session will be concluded with an interactive session discussing the topics described above, as well as a discussion on what Scand-LAS can do for you. We want your opinion – bring your phones!

## Assuring competence in practical tasks- ETPLAS working group 4

6. Focus on: The 3Rs in education

Lucy Whitfield<sup>1</sup>, Andreia Costa<sup>2</sup>, Eirini Fragiadaki<sup>3</sup>, Rafael Frias<sup>4</sup>, **Brian Mphande**<sup>5</sup>, Paul West<sup>6</sup>

<sup>1</sup> Royal veterinary college, UK

<sup>2</sup> University of porto, Portugal

<sup>3</sup> Hellenic pasteur institute, Greece

<sup>4</sup> Karolinska institute, Sweden

<sup>5</sup> Tampere university, Finland

<sup>6</sup> University of oxford, UK

### Introduction

All those working under the Directive 2010/63/EU are required to be trained and supervised until they are competent to carry out procedures alone.

But how do we know if someone is truly competent?

What is the standard for competence and do we all agree about it?

If we agree, then why do we insist on re-training and re-assessing competence of staff every time that they move to a new institute?

Doing so potentially wastes time and resource for both the researcher and the unit staff.

But of course we do need to be certain that they are indeed competent.

What would we need to know in order to trust the assessment that's already been done?

Validity: The assessment was appropriate for the task.

Transparency: The standard that was required for competence is clear to everyone.

Consistency: The standard set for competence in the task remains the same between different assessors and at different times.

### Methods

Using DOPS to make practical assessment more effective.

DPOS: Directly Observed Practical Skills assessment

### Results

ETPLAS working group developed a 'library' of DOPS for assessment of commonly-used practical techniques.

- » Well validated assessment method for practical tasks
- » Used in healthcare setting for many years
- » Trainee is assessed against pre-determined competence criteria
- » A permanent record of trainee's performance

### Conclusion

Set out the criteria that determine competence. These should be:

- » relevant to the task
- » easy to measure
- » available to the trainer, trainee & assessor so everyone is clear about what is the standard required for competence.

## **Models and simulators that can replace use of live animals in education**

6. Focus on: The 3Rs in education

**Adrian Smith**<sup>1</sup>

<sup>1</sup> Norecopa

### **Introduction**

The usefulness of alternatives to animal use is sometimes hotly debated, especially by those who are unaware of the alternatives that are now available. This debate is partly due to confusion between two concepts: fidelity and discrimination. In addition, the value of alternatives must always be weighed against the objectives of the teaching situation. A list of criteria to help decision-making will be discussed during the presentation.

### **Methods**

Norecopa's staff have been collecting information about alternatives and supplements to animal use in education since 1991. This include resources at all levels of education and training, from dissection alternatives for schools, through undergraduate training in subjects such as physiology and pharmacology, to the needs of animal care staff and scientists who will be using animals in their daily work.

### **Results**

This information has been collected in a database, NORINA, which is available free of charge online (<https://norecopa.no/NORINA>). The database is updated continuously, as new resources are produced and old ones go out of production. NORINA curently contains 2,800 products. A large number of search filters can be used to narrow the number of hits to the area of interest and type of alternative (e.g. simulators to practise blood sampling). In addition to commercially available products, there are now many people producing homemade resources. Norecopa held a workshop about these and has collected links to those who are particularly active in this field.

### **Conclusion**

This presentation will showcase examples of a wide range of resources which are relevant to the field of laboratory animal science.

# **7. Focus on: Pain management**

# Acute phase proteins as inflammatory markers of Respiratory Disease (RD) in dairy calves

7. Focus on: Pain management

**Rohish Kaura**<sup>1</sup>, *Elisabeth Dorbek-Kolin*<sup>1</sup>, *Marina Loch*<sup>1</sup>, *Dagni-Alice Viidu*<sup>1</sup>, *Toomas Orro*<sup>1</sup>, *Kerli Mõtus*<sup>1</sup>

<sup>1</sup> Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Estonia

## Introduction

Respiratory diseases (RD) are the leading cause of morbidity, mortality, and antimicrobial consumption of dairy calves worldwide. RD causes pain and distress to young calves through tissue injury and inflammatory changes. Acute phase proteins (APPs) as biomarkers are nonspecific but highly sensitive indicators of inflammation and can reflect the effects of cytokine changes in body objectively. The present study aimed to identify the associations between clinical respiratory disease signs in calves, occurrence of bacterial pathogens in lower respiratory tract and APPs.

## Methods

In total, 150 calves (age range 2-86 days) from fifteen large (>400 dairy cows) Estonian dairy farms were included. Blood samples were taken from five calves suffering from respiratory distress and five apparently healthy calves per herd. From blood samples, haptoglobin (Hp) concentration was measured by using haemoglobin-binding assay, serum amyloid A (SAA) by ELISA kit and fibrinogen (Fib) by heat precipitation technique. Tracheobronchial lavage (TBL) sampling was performed in all calves for bacterial culturing and *Mycoplasma bovis* PCR analysis.

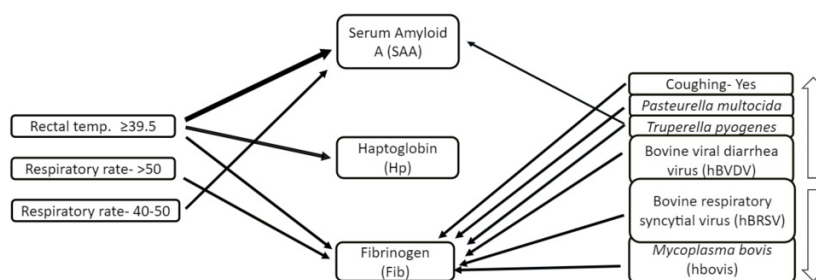
## Results

Results of mixed-effects linear regression analysis of three APPs is presented in the following figure 1.

## Conclusion

The results of this study indicate that clinical signs of RD and presence of bacterial pathogens in lower respiratory tract are associated with APPs. Fibrinogen measurements has the best potential to estimate the severity of RD causing inflammation, thus pain and distress caused to the animals.

This work was supported by the Estonian Research Council grant (PSG268).



**Figure 1.** Mixed effect linear regression analysis investigating the association of APPs with clinical respiratory disease signs and lower respiratory tract pathogens



## Effects of analgesia on functional outcome and well-being of rats subjected to spinal cord injury

7. Focus on: Pain management

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### Introduction

Rats are commonly used in spinal cord injury (SCI) research. Despite the severe injury in the animals produced, the use of analgesia is very low due to concerns about possible confounding effects from the analgesic drugs. This study aimed at investigating the effects of analgesia on the functional outcome and well-being of SCI rats.

### Methods

Forty-two female Wistar rats, (CrI: WI), were randomly divided into six equal groups. A dental burr-assisted laminectomy was performed at the T10-T11 vertebra level, and SCI of high severity was induced using a custom-built force deliverer. Five analgesic regimens (carprofen (5mg/kg); carprofen + buprenorphine (5mg/kg + 0.05mg/kg); meloxicam (1mg/kg); meloxicam + buprenorphine (1mg/kg + 0.05mg/kg); and tramadol + buprenorphine (5mg/kg + 0.05mg/kg)) were administered to the groups and compared to control. During eight weeks of study, Basso Beattie and Bresnahan (BBB) scoring, Novel Object Recognition (NOR) test, von Frey test, and histopathology served as markers of functionality, while body weight changes, Rat Grimace Scale (RGS), and dark-phase home cage activity were used as markers for well-being.

### Results

None of the markers of functionality differed between animals treated with analgesia and control animals. In treated animals, the RGS was lower on days 1 and 7 and the weight gain was higher on days 7, 14, 21, and 28, and activity on days 1, 7, and 14.

### Conclusion

Analgesia had no effects on the functional outcome, but improved animal well-being in SCI rats compared to when no analgesics were used.

# **8. Focus on: Male mouse aggression**

## Aggression in group-housed male mice: a dual approach to increased understanding

8. Focus on: Male mouse aggression

Elin M. Weber<sup>1</sup>, Josefina Zidar<sup>1</sup>, Birgit Ewaldsson<sup>2</sup>, Kaisa Askevik<sup>3</sup>, **Emma Svensk<sup>3</sup>**, Elin Törnqvist<sup>4, 5, 6</sup>

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<sup>3</sup> Swedish 3Rs Center, Swedish Board of Agriculture

<sup>4</sup> Swedish National Committee for the Protection of Animals Used for Scientific Purposes, Swedish Board of Agriculture

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<sup>6</sup> Institute of Environmental Medicine, Karolinska Institutet

### Introduction

Aggression among group-housed male mice is a major animal welfare concern often observed at animal facilities. Pain, stress, and social isolation, as a consequence of aggression, can alter physiological parameters creating variability and jeopardizing scientific validity.

### Methods

The Swedish National Committee for the Protection of Animals Used for Scientific Purposes and the Swedish 3Rs Center collaborated with experts to map the problem of aggression in group housed male mice at university research facilities and private companies in Sweden. Veterinarians, ethologists, animal technicians and researchers participated in eleven workshops and one survey. The results have been communicated at different meetings and compiled in national guidelines. Furthermore, we performed a systematic literature review to map how the literature support, or do not support, published recommendations within the field.

### Results

The compiled results suggest keeping stable groups, using less aggressive strains and enrichment as important factors to prevent aggression. Hiding devices and nesting material seem to prevent aggression better than locomotor devices that could be monopolized. However, there is no one-solution-fits-all and different interventions can work at different facilities. The results from the workshops and survey also highlight the importance of establishing and evaluating routines at the facilities, and to promote collaboration and communication between researchers, veterinarians, animal technicians and the management.

### Conclusion

Both approaches – a literature review and compiled experiences from research facilities, proved to be important and to complement each other in developing guidelines on how to prevent aggression in group housed male mice.

## Housing male mice with ovariectomized females can overcome limitations of male mice group housing in long term studies

8. Focus on: Male mouse aggression

**Lena William-Olsson**<sup>1</sup>, *Anna Thorén*<sup>1</sup>, *Meja Ekholm*<sup>1</sup>, *Daniel Karlsson*<sup>2</sup>, *Bader Zarrouki*<sup>3</sup>, *Birgit Ewaldsson*<sup>1</sup>

<sup>1</sup> Animal Science and Technologies, Clinical Pharmacology and Safety Sciences, Biopharmaceuticals R&D, AstraZeneca R&D, Gothenburg, Sweden

<sup>2</sup> Functional Project Management, Clinical Pharmacology and Safety Sciences, AstraZeneca R&D, Gothenburg, Sweden

<sup>3</sup> Bioscience Metabolism, Research and early development, Cardiovascular, Renal and Metabolism (CVRM), Biopharmaceuticals R&D, AstraZeneca R&D, Gothenburg, Sweden

### Introduction

Wild mice typically form groups with one dominant male, several females, often with offspring, and other subordinate males loosely connected to the group. The dominant male show aggression towards other males, which makes group housing of male mice in laboratory setting problematic. To mimic social conditions and to avoid long term single housing, pairs of male mice and ovariectomized female mice were kept together.

### Methods

In long term studies where bodyweight is an essential read out, and diet intake drives disease progression, all mice must have the same housing conditions to avoid confounding factors, since single housed and group housed mice develop different eating and bodyweight patterns. In this 12 months study, male and ovariectomized female mice were paired together and a scheme was established to closely monitor aggression and other signs of compromised welfare.

### Results

This scheme resulted in no aggressive or dominant behavior from the male mice, with clear signs of compatibility, like nesting together, no deviation in body weights or other negative effects on animal welfare on males or females.

### Conclusion

This scheme must always be preceded by careful considerations of the 3Rs, considering the harm benefit of using ovariectomized females as companion to long term single housed males. In this study the mice formed compatible pairs, pointing towards improved welfare considering socialization factors. However the 3R implication of including animals required to undergo surgery as companions only is an area for discussion, emphasizing the need for potential alternative options to overcome this hurdle.

## Illustrating behavioral epidemiology as a 3Rs solution to complex multifactorial problems: Fighting in mice as a worked example

8. Focus on: Male mouse aggression

**Elin M Weber**<sup>1</sup>, *Charlotte Berg*<sup>1</sup>, *Joseph P Garner*<sup>2</sup>

<sup>1</sup> Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden

<sup>2</sup> Department of Comparative Medicine, Stanford University, Stanford, California, USA

### Introduction

Welfare problems with multifactorial etiologies present a unique 3Rs challenge. They are inherently difficult to manage in practice. Worse still, testing causal factors one-by-one in conventional controlled experiments involves large numbers of animals and often produces contradictory or irreproducible results – particularly for severe but rare problems (like stereotypies, compulsive behaviors and fighting).

### Methods

Behavioral epidemiology is a powerful solution for Reduction, Refinement (multiple factors are investigated and controlled for simultaneously), and Replacement (existing animal populations or medical records are studied). Here we illustrate this approach using our work in mouse aggression. Group housing is fundamental to ensure good animal welfare, but comes at the cost of aggression. Therefore finding ways to reduce aggression is crucial.

### Results

We will discuss three studies. First the largest and most comprehensive study of any behavioral problem in mice – representing over 2500 cages of mice and a full year of data collection. 13.8% of males were observed fighting, and a variety of previously known (e.g. strain), and novel risk factors were identified (e.g. cage position), while other experimental risk factors (including from our own work) were not significant. These will be discussed as examples of the strengths and weaknesses of the technique.

In a pilot study, the same protocol was used to collect data in a Swedish mouse facility. Lessons learned from this pilot, particularly in terms of translating from USA to Swedish husbandry norms, will be discussed.

### Conclusion

Informed by this work, we are currently conducting a full-scale epidemiological study including several Swedish research facilities.

# **9. Focus on: Monitoring animals under anesthesia**

# Monitoring animals under anesthesia in the wild

9. Focus on: Monitoring animals under anesthesia

**Åsa Fahlman**<sup>1</sup>

<sup>1</sup> SLU Swedish Biodiversity Centre, Uppsala, Sweden

## **Introduction**

Capture and anesthesia of free-ranging wild animals are often carried out under the most challenging conditions. The capture event as well as the anaesthetic drugs influence physiological parameters of the animals. It is important to minimize stress for the animal and ensure stable physiology during anesthesia. Thus, evaluation and monitoring of anesthesia are crucial to ensure wildlife welfare and minimize the risk of morbidity and mortality. Portable battery-driven monitoring devices and blood analysers enable in-depth monitoring in field situations. Since wild animals commonly develop hypoxemia during anesthesia, oxygen therapy is an essential and simple tool to improve safety, which can be provided by oxygen cylinders or portable oxygen concentrators. I will share experiences, practical considerations, and research results from over 20 years of wildlife work in Africa, Asia, North America and Scandinavia for improved capture, anesthesia, and monitoring, as well as prevention and treatment of complications.

## **Methods**

## **Results**

## **Conclusion**

# 10. Focus on: Refinement



# Discovering stress-free biomarkers in various mouse models using digital ventilated cages (DVC®) technology

10. Focus on: Refinement

**Giorgio Rosati**<sup>1</sup>

<sup>1</sup> Tecniplast SpA

## Introduction

Implementing home cage monitoring systems facilitates the potential for novel discoveries by utilising a 24/7, non-stressful environment for study animals. Recent distinctions have been made between traditional "benchtop technologies," in which animals are limited to brief exposures in experimental environments, and "real home cage technologies," in which animals spend most of their lives.

## Methods

Of these real-home cage technologies, digital ventilated cages (DVC®) represent a viable and scalable option, as they allow for simultaneous monitoring of 1-1000+ cages, permit the study of animals without modifying their housing conditions, and enable the incorporation of environmental enrichment through the utilisation of micro electromagnetic field technology.

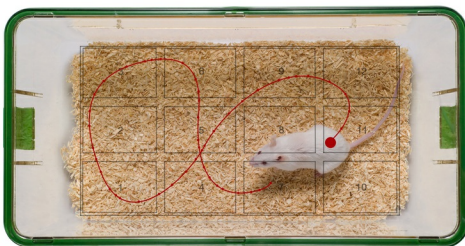
## Results

In this presentation, we will provide examples of research applications utilising DVC® technology, such as:

- the examination of polyuria as a marker of sustained hyperglycemia in diabetes
- identification of previously unobserved fighting events in group-housed animals
- detection of early indicators of welfare issues in the most used mouse model of Covid-19

## Conclusion

Overall, the DVC® technology represents an advancement in refinement and reduction of animal use by providing 24/7 monitoring in a scalable manner while promoting animal welfare and enhancing the characterisation of study models.



## Evaluation of 4 different nesting materials for laboratory mice

10. Focus on: Refinement

**Anna Österlund<sup>1</sup>**, *Linda Kroon<sup>1</sup>, Jasmine Ivarsson<sup>1</sup>, Liselotte Hallengren<sup>1</sup>*

<sup>1</sup> Animal Science and Technologies, Clinical Pharmacology and Safety Science, Biopharmaceuticals R&D, AstraZeneca R&D, Gothenburg, Sweden

### Introduction

The aim of the study was to compare 4 different nesting materials for mice. :1 Bed-r  
Nest, 2 Paper wool, 3 Sizzle Nest and 4 Sizzle Nest fine, all provided by Datesand/Scanbur.

### Methods

During 4 weeks of study time, 4 different groups of mice (pregnant females and females with pups, group housed females, single housed males and group housed males) had access to all of the nesting materials, one at a time, for one week. At weekly cage change the nest was scored and thereafter replaced with new, different material.

### Results

None of the materials has caused any injuries or trapping in the animals. All nesting materials were used for nest building. The paper wool got the highest score with 181 points, followed by Sizzle nest fine with 178 points, sizzle nest with 175 points and and Bed  
Nest with 168 points.

### Conclusion

All mice were using the nesting materials for nestbuilding, with very few exceptions. In the cases where one of the new nesting materials was not used, the animals did build nests when given the other nesting materials the following week, indicating that there was a slight preference for the softer materials as the low scores only occurred with the harder materials.



## **Norecopa: PREPARE for Better Science**

10. Focus on: Refinement

**Adrian Smith**<sup>1</sup>

<sup>1</sup> Norecopa, % Norwegian Veterinary Institute, P.O. Box 64, 1431 Ås, Norway

### **Introduction**

The main goal of Norecopa (norecopa.no) is to advance the 3Rs by consensus between all stakeholders. One of Norecopa's main resources is its website, which currently contains over 9,000 pages with links to global 3R resources.

### **Methods**

Since the last Scand-LAS meeting, the following resources that are of interest outside Norway have been produced or updated:

- The PREPARE guidelines for planning animal experiments consist of a 2-page checklist and a website with links to resources for each topic. The checklist is now available in 34 languages and three versions (pdf and electronic) and the website now includes additional tabs for fish researchers.
- Norecopa's Refinement Wiki offers a forum for rapid publication of suggestions on how to refine animal experiments.
- A comprehensive slide deck describing the development and use of the 3R concept has been produced.
- A global interactive map of 3R centres and lab animal science associations and networks has been launched.

### **Results**

The software, search engine and search filters have also been upgraded. The website currently attracts approx. 1,000 hits per day.

### **Conclusion**

The Norecopa website aims to be the most comprehensive global overview of 3R resources. Newsletters issued 7-8 times a year provide quick updates of the latest advances in this field.

## Refined management and care of newborn Göttingen minipigs

10. Focus on: Refinement

**Tania Panfilova**<sup>1</sup>, *Carina Anker*<sup>1</sup>, *Maja Ramløse*<sup>1</sup>, *Kirsten Rosenmay Jacobsen*<sup>1</sup>

<sup>1</sup> Ellegaard Göttingen Minipigs A/S

### Introduction

Through early intervention immediately after birth it is possible to reduce the mortality rate among and improve the welfare of newborn piglets. Good results can be achieved through refinement of

common procedures in the farrowing stable and for the newborn piglets in e.g. juvenile toxicology and safety studies. This can be through measures like administration of colostrum to weak-born piglets,

keeping the piglets warm, and sports tape on the forelegs to reduce the occurrence of leg wounds sustained during suckling. Furthermore, socialization of the sow before and after farrowing creates a

calm and trusting environment even when staff members are interacting with the piglets immediately after farrowing. An optimized program for the care of newborn piglets will improve the health and

welfare for the animals, thus, building a solid foundation for the minipigs to grow and thrive.

### Methods

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### Results

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### Conclusion

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## Swedish acclimatization guidelines for rats and mice

10. Focus on: Refinement

Viktoria Brånsgård<sup>1</sup>, Frida Karlsson<sup>1</sup>, **Emelie Jansson**<sup>2</sup>, Elin Weber<sup>3</sup>, Elin Spangenberg<sup>4</sup>, Katarina Cvek<sup>5, 6</sup>, Erika Roman<sup>1, 5, 7</sup>

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<sup>3</sup> Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden

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<sup>5</sup> Swedish National Committee for the Protection of Animals Used for Scientific Purposes, Swedish Board of Agriculture, Jönköping, Sweden

<sup>6</sup> Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>7</sup> Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

### Introduction

Acclimatization after transport is commonly used to reduce stress after e.g. transport to a new facility, and required in the Directive 2010/63/EU. However, details on how to acclimatize animals are not provided and there is a need for harmonized guidelines to improve scientific comparison.

### Methods

As a first step towards formulating guidelines for acclimatization of rats and mice, the Swedish 3Rs Center, together with veterinary students, sent out an anonymous survey to Swedish research facilities, investigating how, when and for how long they acclimatize their rats and mice.

### Results

Preliminary results indicate large differences among Swedish research facilities. Most respondents (91%) answered that rats and mice are acclimatized after transportation to the facility. However, the acclimatization period ranged anywhere between 3 days to 4 weeks, with most respondents indicating 5-7 days or around two weeks. The majority (53%) answered that there are written instructions for acclimatization within their organization, the rest answered no (13%) or do not know (34%). When asked if animals are acclimatized after other changes to their daily routines, most respondents answered that they do not acclimatize or do not know if acclimatization occurs when animals are being moved within the facility (75%), experiencing changes to the circadian rhythm (80%) or being regrouped (86%).

### Conclusion

The survey shows that guidelines are needed to harmonize acclimatization of research animals in Sweden. Together with experts, we will compile scientific evidence with the survey results, to create a guide of best practice on acclimatization of mice and rats in experiments.

## The mouse in the house - an alternative to tail handling

10. Focus on: Refinement

Julia Wallin<sup>1</sup>, *Elin M Weber*<sup>1</sup>

<sup>1</sup> Department of animal environment and health, Swedish university of agricultural sciences

### Introduction

Worldwide, mice are the most used animal in biomedical research, and it is well known that laboratory staff's handling techniques affect the welfare of the mice. Picking mice up by their tails causes aversive behaviour and anxiety, but using a tunnel reduces both. This study tested whether using another object to lift mice from their home cage could produce similar results to a tunnel.

### Methods

In this study, 36 CBB (C57xBALB) mice were picked up either by the base of the tail or by an upside-down house. Willingness to interact with the assigned handling device was observed, and anxiety and exploration was tested for in an open field arena.

### Results

Preliminary results indicate that mice handled with the house were more willing to approach and stay on the device than those handled by the tail. During each test session, the house handled group increased their interaction time after the first interaction. The house handled mice also showed lower anxiety, spending more time in the centre and middle areas of the open field while the tail handled mice spent more time in the outer area. During handling, tail handled mice were observed to urinate, defecate and vocalise on 48 occasions in total, compared to one instance of defecation by the house handled mice.

### Conclusion

This study indicates that using a house to pick up mice reduces anxiety and aversion behaviours that are commonly seen in mice picked up by their tails.

## **We do not need to, and should not, fast our animals before the sucrose preference test for depression**

10. Focus on: Refinement

**Jenny Berrio**<sup>1</sup>, *Sara Hestehave*<sup>2</sup>, *Otto Kalliokoski*<sup>1</sup>

<sup>1</sup> Department of Experimental Medicine. Section of Research and Education. Faculty of Health and Medical sciences. University of Copenhagen.

<sup>2</sup> Department of Cell and Developmental Biology. Division of Biosciences. Faculty of Life Sciences. University College London

### **Introduction**

A common model of depression exposes rodents to weeks of daily but varied unpredictable stressors in order to mimic the chronic stress of daily human life that is linked to depression. This model is validated with the sucrose preference test, a simple test that measures how much a sweetened solution is preferred over water. The assumption is that this preference is in proportion to the pleasure experienced by consuming something sweet. Since depression decreases the ability to feel pleasure, stressed rodents that consume less sweetened solution than unstressed peers are considered depressed.

### **Methods**

While the theory seems straightforward, the results are not. Contradictory results across studies have concerned researchers for decades. A possible suspect is the common practice of fasting animals for long periods (12-24 hours) before the test, which introduces hunger and thirst into the equation. After systematically analysing over a hundred studies that avoided such practices, we made two important findings.

### **Results**

One: fasting is not only unnecessary but also inadvisable. Even in the absence of long fasting, we found evidence in support of additional non-pleasure related factors driving sweet consumption; evidence we interpret as relating to metabolism. Two: strategies meant to reduce bias when conducting the test were often missing and reporting was often unclear and incomplete.

### **Conclusion**

Improving the methodological rigor and addressing confounding factors in the test prevents misleading conclusions that harm the translatability of the research and perpetuates the use of animals for little gain. A first step is to remove altogether fasting before sucrose preference testing.

# 11. Focus on: Reduction



## Please stop using and teaching the Resource Equation

11. Focus on: Reduction

**Otto Kalliokoski**<sup>1</sup>

<sup>1</sup> The Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

### **Introduction**

With a number of initiatives focused on improving the experimental design of animal studies there has been a renewed interest in properly dimensioning experiments. The question “how many animals should I use in my study?” should never be settled through guesswork. Our gut feelings should not enter the picture. But because methods focusing on statistical power can be complicated and inconvenient we have seen an uptick in the use of other methods; some of which are ill-suited for the task.

### **Methods**

An alternative method taught in many textbooks, and one which is explicitly mentioned in the learning objectives for FELASA courses (function B), is Roger Mead’s Resource Equation. I will demonstrate that this method has no statistical basis. It was never conceived as a method for obtaining sample sizes and it will recommend inappropriately small experiments, consistently leading to underpowered studies.

### **Results**

Software-aided methods for creating appropriately-sized experiments will outperform the Resource Equation at every turn, since the latter will only create properly powered studies through sheer luck. We can demonstrate this, empirically, using computer simulations and previously obtained data from properly conducted animal studies.

### **Conclusion**

We need to stop using the Resource Equation to dimension our studies. We should not accept it as a justification for experimental designs when applying for ethical approval; we should not accept it in journal articles; and we should definitely stop teaching it as a legitimate alternative to statistically sound methods. Its use leads to a waste of animal lives and it compromises the associated science.

# **12. Focus on: Culture of care at the vendors**

# Dry Heat Sterilization in Vivariums - An Alternative Option for Sterilization in the Lab Animal Facility

12. Focus on: Culture of care at the vendors

**Robert Davis**<sup>1</sup>, *Tine Jensen*<sup>1</sup>

<sup>1</sup> Opend ApS, Denmark

## Introduction

The presentation focuses on the use of dry heat, instead of steam, for the sterilization of laboratory animal cages, IVC racks, enrichment and other items used in the vivarium. The outline of the talk is as follows:

- Development of dry heat sterilization in laboratory animal facilities
- What is dry heat sterilization
  - How does it work with laboratory animal cages, IVC racks and related items
- How is the dry heat sterilizer configured
- What are the benefits of dry heat sterilization
- What are the limitations of dry heat sterilization
- (8) recent case studies showing specific customer challenges and how dry heat sterilization solved them
- How does dry heat sterilization compare to steam sterilization
- How are these systems validated

## Methods

## Results

## Conclusion

## Implementing change- creating a stimulating and cooperative environment for Göttingen Minipigs

12. Focus on: Culture of care at the vendors

**Maja Ramløse<sup>1</sup>**, *Kirsten Rosenmay Jacobsen<sup>1</sup>*, *Carina Anker<sup>1</sup>*, *Susi Søgaard<sup>1</sup>*

<sup>1</sup> Ellegaard Göttingen Minipigs A/S

### Introduction

Creating and maintaining a good Culture of Care and working with 3R is a continuous task with many positive outcomes: Better science, better animal welfare and better working terms for the staff. Both regular housing conditions as well as project set-ups benefit from being designed with these principles in mind.

Göttingen Minipigs are highly intelligent animals exhibiting complex behaviours. They have specific needs which must be met to ensure a stimulating and appropriate environment and overall good animal welfare. Accommodating the needs for which the minipig has a strong motivation, has many physiological and behavioral benefits such as reducing the incidence and/or severity of undesirable or abnormal behaviours. At Ellegaard Göttingen Minipigs we provide species-specific enrichment that enable expression and stimulation of natural needs such as rooting and social behaviour, e.g. with ice blocks and play rooms. Other initiatives, such as socialization and positive human interactions serve not only as enrichment but also promote a calm and cooperative environment during studies. This can be performed through positive reinforcement training, where Göttingen Minipigs have proved excellent candidates with a wide range of training options for both general husbandry and study related procedures.

Systematization is a valuable tool when implementing the above. Furthermore, staff commitment and a mindset that challenges current standards of e.g. housing and interactions is key when working with 3R and generating a good Culture of Care. In our experience, these are promoted by encouraging staff input and involvement when implementing new initiatives and standards.

### Methods

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### Results

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### Conclusion

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# Invited speakers

# A standardised score sheet template for assessing rodent health

Invited speakers

**Elin Spangenberg**<sup>1</sup>, *Patricia Hedenqvist*<sup>2</sup>, *Emelie Jansson*<sup>3</sup>, *Erika Roman*<sup>4, 5, 6</sup>

<sup>1</sup> Swedish Centre for Animal Welfare, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>2</sup> Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>3</sup> Swedish 3Rs Center, Swedish Board of Agriculture, Jönköping, Sweden

<sup>4</sup> Swedish National Committee for the Protection of Animals Used for Scientific Purposes, Swedish Board of Agriculture, Jönköping, Sweden

<sup>5</sup> Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>6</sup> Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

## **Introduction**

Score sheets formalise and standardise the assessment of welfare and make it possible to record the impacts of scientific procedures. The Swedish National Committee for the Protection of Animals used for Scientific Purposes and its group of experts has worked out a score sheet template for assessment of rodent health.

## **Methods**

The template is based on existing score sheets and further developed with advice from veterinarians from Swedish research institutions. The score sheet was published by the Swedish 3Rs Center for the purpose of national standardisation.

## **Results**

The template can be used to assess animals' health before, during and after a study to estimate if the animals' health has deteriorated, as well as to decide if the humane endpoint is reached. It can be used by the principal investigator to set the humane endpoint in a study. The template lists several categories of factors to assess, including general condition, condition of the fur, skin and teeth and breathing. Each parameter is scored 0: no change, 1: slight change or 4: substantial change from normal. If the criteria for the humane endpoint is reached, the experiment should be stopped and the animal treated or euthanized, regardless of whether the purpose of the study has been achieved or not.

## **Conclusion**

Our template has been shared nationally and several facilities have started to use it to assess the health of their rodents.

# Assessing animal welfare - the familiar and the 'exotic'

Invited speakers

**Oliver Burman**<sup>1</sup>

<sup>1</sup> Animal Behaviour, Cognition & Welfare Research Group, University of Lincoln.

## **Introduction**

Although there are many existing ways to assess the welfare of different animal species, some of the available welfare indicators have limitations, such as ambiguity in how they are interpreted, or may fail to generalise across species. This means there remains a need to identify new ways of assessing animal welfare - even for those familiar species that are well-researched. In contrast, 'exotic' animals such as reptiles, that are increasingly common as pets and have important welfare concerns, have been the focus of relatively little research, and so there are correspondingly few established measures of welfare. For these less familiar 'exotic' species the primary focus is therefore to explore the efficacy of existing animal welfare indicators.

## **Methods**

In this presentation I will give an overview of two themes in my research into welfare assessment: (1) *Developing new welfare measures in familiar species*; and (2) *Extending familiar welfare measures to exotic species*.

## **Results**

I will include examples of research investigating the use of novel cognitive and behavioural welfare indicators in dogs and fish, as well as the use of more familiar welfare indicators in exotic species (tortoises, lizards & snakes). Finally, I will briefly describe some more applied studies that use combinations of these different measures alongside other approaches to welfare assessment (preference testing) to address key housing/husbandry issues.

## **Conclusion**

I hope that this talk will demonstrate how we can move towards more effective welfare assessment, and thus a better understanding of how to improve captive animal welfare, across a diverse range of species.

## **Behavioral profiling of rats, mice and zebrafish**

Invited speakers

**Erika Roman**<sup>1</sup>

<sup>1</sup> Department of Pharmaceutical Biosciences, Uppsala University & Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences (SLU)

### **Introduction**

Increased understanding of complex behaviors may demand for new approaches. Conventional behavioral tests offer limited possibilities to capture a broader behavioral repertoire, and if combined into test batteries there is a risk of carry-over effects.

### **Methods**

The multivariate concentric square field™ (MCSF), originally developed for rats, is unique in its design by provoking behaviors associated with exploration, risk taking and shelter seeking. Thereby a behavioral profile is generated in a single session.

### **Results**

In this presentation, examples of the use of the MCSF test for behavioral profiling of rats, mice and zebrafish will be presented.

### **Conclusion**

The MCSF test may constitute an important complement to conventional tests used in preclinical research.



# BEST ANAESTHESIA PROTOCOL FOR FISH SPECIES AND SITUATION

Invited speakers

**Albin Gräns**<sup>1</sup>

<sup>1</sup> Department of Animal Environment and Health, Swedish University of Agricultural Sciences

## **Introduction**

In this project, we determined how different anaesthetics affect the health and welfare of fish and to what extent are the effects context dependent. We aimed to determine the best anaesthetics for three selected model species (*i.e.* zebrafish, brown trout and rainbow trout), but the results will be valid for a range of fish species.

## **Methods**

Here we conducted a series of studies covering the induction time of unconsciousness, different drugs, to the ultimate consequences for animals being released back into their respective home environments. We used a range of state of the art methods; including non-invasive technique to record electroencephalogram (EEG) and Electrocardiography (ECG). For fish released into the wild, growth and survival was complimented with long-term measurements of heart rate and body temperature using biologgers to follow the recovery process.

## **Results**

This is the first comprehensive evaluation of various common anesthetic compounds using recordings of EEG and ECG. We show that the induction to narcosis differs between compounds in terms of required time and dose, and that there is substantial variations both between and among species.

## **Conclusion**

Results and conclusions will be presented and discussed in relation to our three respective model species.

## **Cage change on demand, challenges with implementation**

Invited speakers

**Rebecca Sandgren<sup>1</sup>**

<sup>1</sup> none

### **Introduction**

Standard rodent husbandry often includes a weekly cage change. Cage change is done to provide the animals with a clean environment, however, the clean and new environment effects the animals and can cause stress. Therefore it is beneficial to find a balance where time between cage change is as long as possible without the cage environment effecting animal welfare. I will present the challenges we encountered at Lund University in our work with implementing "cage change on demand", including challenges with setting new criteria for cage change and adapting husbandry routines.

### **Methods**

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### **Results**

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### **Conclusion**

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## Clinical training center

Invited speakers

**Isabelle Werner**<sup>1</sup> , *Desirée Karlsson*<sup>1</sup>

<sup>1</sup> Swedish University of Agricultural Sciences

### **Introduction**

Our group consists of six members and we help prepare students for their future career in animal health care, and provide assistance during animal research. Both veterinarians and veterinary nurse students use our simulated models and our facilities to practice and improve their skills. Our vision is to develop new ways for students to learn animal healthcare, without doing animals' unnecessary harm. KTC prioritizes animal welfare by using simulated models, as our primary method for training, instead of laboratory animals.

Our facility has a variety of different training animals at our disposal, everything from life-size cow models to fake rats. We strive to be flexible to the needs of the students. They are welcome at almost any time to practise on their own, and as many times as they like. When students practise drawing blood samples, we use different training models instead of animals. To our disposal, we have a lot of expired equipment that the students can use.

Before moving on to live animals, veterinary nurse students must perform a clinical exam to make sure they know the strict rules of hygiene and safety. They have to show they possess the theoretical knowledge, the right routines and know how to use the tools properly.

We also have euthanized patients given by their owners for exercises in operation and showing anatomy.

### **Methods**

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### **Results**

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### **Conclusion**

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# Culture of Care and 3Rs in practice- How to make people and organisations tick

Invited speakers

**Marie Hornfelt**<sup>1</sup>

<sup>1</sup> Sahlgrenska Academy, University of Gothenburg

## **Introduction**

It is increasingly recognised that nurturing a Culture of Care in animal research organisations, could benefit animal welfare and 3Rs, the quality of science as well as the wellbeing of the people in it. Scientific knowledge and understanding of animal welfare are also constantly evolving, providing immense opportunities for advancing the 3Rs. However, increased pressure on animal research organisations to run lean and cost-effective operations and a strong competing pressure in the research ecosystem, make it difficult to secure resources and engage scientists to establish new 3Rs approaches. How can these hurdles be overcome and what are key factors in creating a Culture of Care that recognises 3Rs opportunities and put them into practice? What makes people and organisations tick?

This presentation will focus on the strategy and framework that we have established within our organisation at the University of Gothenburg, to create and support a Culture of Care and promote the 3Rs. With a clear aim of strengthening leadership and empowerment, driving continuous improvement, and creating a supportive and collaborative working environment. The framework includes platforms for communication and collaboration between scientist and the laboratory animal science organisation as well as dedicated resources for 3Rs and competence development. I will share key milestones and recent examples of our 3Rs-initiatives, the learnings that we have made as well as some of the challenges we are facing.

## **Methods**

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## **Results**

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## **Conclusion**

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# Culture of care in the wild - does anyone care?

Invited speakers

**Jonas Malmsten**<sup>1</sup>

<sup>1</sup> Department of Wildlife, Fish & Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden

## Introduction

Research in wild mammals has been around for decades in the Nordic countries. Ranging from mice to moose, animals have been caught, immobilized, handled, sampled, and killed in a number of ways of which some are highly questionable. With the exception of bats, more than 50 different species of mammals are being used or have been used over the years, and each species or group of species are caught, handled, and dealt with differently. To complicate things even more, the animals are roaming freely in the great outdoors, in different environments, are hard to find, and can never be properly examined prior to capture... Seemingly, research in the different species are connected to certain individual researchers or groups, which can affect the culture of care (CoC). Still, CoC is there in different ways, although not explicitly explained or expressed. There are examples of positive and negative sides to this, which will be addressed in this presentation.

## Methods

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## Results

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## Conclusion

Although highly skilled and experienced, few wildlife researchers seem to be aware of the concept of CoC. This does not necessarily imply that such a culture is non-existent. As in many other fields, these things are people dependent, but winds of change are blowing which will have positive impacts on animal welfare, as well as on the well-being of the people involved in the work.

Photo: Remote chemical capture of moose from a helicopter on Isle of Öland



# Culture of Care - What do we mean and how do we achieve the benefits?

Invited speakers

**Lotte Martoft**<sup>1</sup>

<sup>1</sup> Aim2Achieve

## **Introduction**

Culture of Care (CoC) was introduced to the laboratory animal community with the EU Directive 2010/63/EU. Since then, the concept of culture of care with a more focused commitment to animal welfare, care of staff, transparency, and scientific quality has become well-known to the research animal community. Activities and recommendations aiming to promote and facilitate CoC improvements are provided by many stakeholders from EU, national 3Rs centres, professional societies and animal welfare associations. This is a great help, but even so, we often struggle in our efforts to translate the concept into an effective strategy to improve CoC at the workplace level.

This talk will explore the value of driving CoC improvements based on my experiences from working in animal research in AstraZeneca. It will also highlight some of the most common blockers of CoC advancement, provide examples of open access resources and ideas on how to structure the approach to CoC improvements at the institutional and workplace level.

## **Methods**

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## **Results**

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## **Conclusion**

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## Designing and running a zebrafish facility

Invited speakers

**Lars Bräutigam**<sup>1</sup> , *Chiara Zullian*<sup>1</sup>

<sup>1</sup> Comparative Medicine, Karolinska Institutet, Stockholm

### Introduction

Are you working with zebrafish or are you planning to establish a new facility? Then this workshop might be especially interesting for you!

Lars Bräutigam, the head of the zebrafish core facility at Karolinska Institutet and Chiara Zullian, the designated veterinarian, will lead a workshop for designing, running, and working in a zebrafish facility.

The main topics of the workshop are:

- Designing a zebrafish facility that allow for optimal workflows
- Technical requirements such as ventilation, electricity, and water supply
- Biosecurity in zebrafish facilities
- Raising and breeding zebrafish
- Physical, social, and sensory enrichment in zebrafish facilities
- The importance of animal welfare for generating reproducible scientific results

The zebrafish core facility at Karolinska Institutet was founded in 2003 and has moved into their new localities in 2020. The facility is the largest zebrafish core facility in the Nordic countries and seven full-time staff serve more than 65 internal, external and corporate user groups.

### Methods

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### Results

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### Conclusion

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# Designing your own cardboard mouse house

Invited speakers

**Jenny Bendtsen**<sup>1</sup>

<sup>1</sup> University of Gothenburg

## **Introduction**

In year 2019, we reviewed all enrichment plans for animal species that are housed in our vivarium within the University of Gothenburg. One clear goal for the mouse enrichment was to provide additional opportunities for the animals to hide, to allow them to exert their natural behaviour. Another aim was to standardise the enrichment material, which at the time included *e.g.* empty toilet rolls and glove boxes, with a undefined chemical content and required a labour-intensive handling process. As we found the different commercially available mouse hideouts (*i.e.* houses or tunnels) cost-ineffective but were determined in our goal of providing our mice with improved enrichment, we initiated a project that resulted in the development of a new cardboard mouse house that is now in use in our vivarium.

The project journey of the cardboard mouse house is the topic of this talk, where the aim is to hopefully inspire others to think outside the box when encountering obstacles regarding refinement and enrichment. In a reality of limited financial resources within academia, we want to show that cheaper solutions can be identified if we invest some time and effort in collaborative welfare projects. We have now designed and produced our very own cardboard house, in collaboration with a small packaging company. The cardboard mouse house we have developed is cheaper than commercially available hideouts and is made of material completely free from plastic, glue, dyes or other added chemicals.

## **Methods**

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## **Results**

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## **Conclusion**

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# Effective pain management in laboratory animals

Invited speakers

**Klas Abelson**<sup>1</sup>, *Carsten Grøndah*<sup>2</sup>

<sup>1</sup> Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup> Veterinary Department, Copenhagen Zoo, Copenhagen, Denmark

## Introduction

Animal experimentation often involves procedures that may inflict pain and pain-induced stress on the animals. This may be a significant cause of suffering in the animals, and may introduce confounding variables leading to less accuracy and precision in the experimental readouts. Hence, for moral as well as scientific reasons, eliminating or minimizing pain is essential.

However, pain management in laboratory animals is neither an easy nor a straightforward task. Assessment and recognition of pain in animals, rodents in particular, is difficult and time consuming and thus the validation of an effective pain treatment is challenging. Besides, the risk of skewing experimental data due to potential confounding effects of the analgesic drugs used must also be taken into consideration and eliminated.

This session will give an introduction to pain and nociception in animals, and present and discuss different strategies for a successful and effective pain management in both rodents and large animals. Recent updates on various drugs and regimens, methods for drug delivery, adequate assessment and recognition of pain and stress, as well as strategies for avoiding negative effects on the scientific validity from the analgesic treatment will be presented and discussed in detail.

## Methods

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## Results

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## Conclusion

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# **Establishment of a searchable database for sharing tissues from experimental animals**

Invited speakers

**Leif Carlsson**<sup>1</sup>

<sup>1</sup> Umeå Centre for Comparative Biology (UCCB). Umeå University. 90187 Umeå. Sweden

## **Introduction**

The concept of the 3Rs (reduce, refine and replace) has to be considered by all scientist using animals in their research. An important contribution to the 3Rs would be if scientist could share tissues from experimental animals. This would also lead to a more efficient use of experimental animals since more information could be retrieved from each individual animal.

## **Methods**

As a first step towards a system where scientists can share tissues from experimental animals, Umeå University has established an informatic system where researchers can upload their samples with all its associated data (tissue type, strain, sex, genotype etc), and search for other tissues for their own research.

## **Results**

This concept aims to show the viability of implementing a common animal tissue catalogue that could be available for the scientific community.

## **Conclusion**

Such catalogue will contribute to a reduction of the number of animals used in research, as well as boosting collaboration between institutions and countries.

## Focus on novel approaches: Home cage monitoring of mice

Invited speakers

**Vootele Voikar**<sup>1</sup>

<sup>1</sup> Neuroscience Center, Helsinki Institute of Life Science, University of Helsinki, Finland

### Introduction

Mice are the most commonly used laboratory animals in biomedical research. Assessment of behavioral phenotype is crucial for evaluation of disease models. Traditionally, it is carried out by using a variety of conventional testing methods where animals are removed from their home cage and placed in dedicated arenas. In addition, monitoring the animal welfare relies mostly on cage-side observations. In both situations, the duration of observation is usually very short and occurring in most facilities during the light phase of the circadian cycle, thus rendering a high possibility for missing the subtle changes. Moreover, behavioral testing can be affected by stress (handling, moving to unfamiliar arenas) and bias. Overall, serious concerns have been expressed regarding the validity and reliability of such measurements. In order to overcome at least some of these concerns, there are various technologies available for automated and continuous monitoring of the animals in their home cages where behavioral and physiological parameters can be recorded. In 2021, pan-European network of researchers started a 4-year COST Action “Improving biomedical research by automated behavior monitoring in the animal home-cage” (CA20135 TEATIME), where experts from different fields joined the forces to critically assess the potential of available technologies, to develop the guidelines and identify the needs for further technological development, including analysis of big data. Short overview of the progress made by the Action during 1,5 years will be provided in this presentation. For more information, please visit <https://www.cost-teatime.org/>.

### Methods

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### Results

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### Conclusion

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## Learning from failure- fostering the culture of care

Invited speakers

**Sofia Östman**<sup>1</sup>

<sup>1</sup> Sofia Östman, Animal Sciences and Technologies, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Gothenburg, Sweden

### **Introduction**

Working with animals in research entails commitment to the Culture of Care (CoC). In AstraZeneca we have pledged to support CoC going above and beyond what is legislatively required of us in animal welfare, care of staff, transparency, and scientific quality. Focusing on errors is closely linked with staff psychosocial security, but also a natural component of securing sustained and improved animal welfare. Talking openly about what goes wrong can be a challenge for staff as it entails sharing of examples and situations of “near misses” or “failures”. To effectively work with learnings from errors demands a pre-established culture of trust and care where staff willingly can share information on things that did not go the way it was planned without the fear of retaliation.

### **Methods**

In this talk I will present the journey of setting up a “No-Blame, Learning from Failures” system in AstraZeneca. To do this, we adopted the “Human and Organizational Performance” (HOP) mindset from workplace safety and used it in the context of culture of care. We developed a “learning-log” and a method to address and take learnings from errors effectively.

### **Results**

The log is an automated process that includes an easily accessible event reporting tool called “Learning Log” and a Root Cause Analysis practice.

### **Conclusion**

I will discuss our learnings (pros, cons, requirements) and provide advice on how to address this new way of working in trustful collaboration with staff.

# Modelling the child eye cancer retinoblastoma using chicken embryonic retina

Invited speakers

**Finn Hallböök**<sup>1</sup>, *Dardan Konjusha*<sup>1</sup>, *Minas Hellsand*<sup>1</sup>, *Hanzhao Zhang*<sup>1</sup>, *Maria Blixt*<sup>1</sup>, *Henrik Ring*<sup>1</sup>

<sup>1</sup> Dept. Immunology, Genetics and Pathology, Uppsala University

## Introduction

Retinoblastoma is a rare, fatal if untreated, childhood cancer of the developing retina with a neuronal origin. The majority of the cancers have bi-allelic RB1 mutations but some types instead have MYCN gene amplifications and it is debated if MYCN is sufficient to drive carcinogenesis without RB1 mutations. The oncogenesis may instead depend on the specific cytogenetic context of the cell origin. We studied if MYCN could drive neoplasia and we mapped the cytogenetic pathways in the cancer cell origin.

## Methods

Embryonic day 3.5 chick retina was transformed by MYCN. Genome integrating MYCN-GFP piggyBac vectors were injected subretinally and the retina was electroporated *in ovo*. Analyses using histology, RNA single-cell seq. and orthotopic injections of established cell lines were performed.

## Results

MYCN transformed chick retina developed tumours at high frequency, expressing a cone photoreceptor progenitor phenotype that was resistant to apoptosis in contrast to other celltypes. Cells proliferated beyond the normal post-mitotic states and formed metastatic cancer that infiltrated optic nerve and sclera. The tumour was classified as anaplastic retinoblastoma. Expression profiling confirmed a cone origin and identified among several pathways augmented CDK-E2F signalling possibly circumventing RB1-deficiency. E2F could be targeted with specific inhibitors that normalized the neoplasia.

## Conclusion

MYCN is sufficient to drive retinoblastoma carcinogenesis in chicken retina. It has the same origin as RB1-deficient retinoblastoma: in the cone photoreceptor lineage. The cone-rich chicken retina is a suitable model while mouse rod-driven cone-poor retina is less good. The augmented E2F signalling in MYCN-driven retinoblastoma was identified as a potential new drug target.

## Monitoring long-term anesthesia in pigs

Invited speakers

**Miklos Lipcsey**<sup>1</sup>

<sup>1</sup> Uppsala university

### **Introduction**

n/a

### **Methods**

n/a

### **Results**

n/a

### **Conclusion**

Anesthetizing animals for hours to days poses challenges in maintaining hemostasis. The aims of anesthesia are to provide optimal conditions for experimental research without suffering by achieving loss of consciousness, analgesia, inhibition of autonomic reflexes, immobilization, and in non-terminal experiments amnesia. Anesthesia is usually achieved by a combination of drugs such as hypnotic and analgetic agents. However, taking over the control of vital organ systems means that these have to be maintained requiring specific knowledge about the physiology of the laboratory animals as well as the pharmacology of the used substances. Clinical monitoring i.e. being continuously attended by staff who can identify signs of stress or deterioration in vital sign is important. In larger animals such as pigs and sheep human monitoring equipment can be used to assess respiration, circulation, level of consciousness, renal function, body temperature.

# Monitoring pain in zebrafish

Invited speakers

**LYNNE SNEDDON<sup>1</sup>**

<sup>1</sup> University of Gothenburg

## **Introduction**

Zebrafish have become an increasingly important model over a wide range of experimental contexts. Routine procedures such as fin clipping, tagging, surgery and exposure to low pH chemicals result in tissue damage that gives rise to pain. To safeguard the welfare of zebrafish it is important that we have a means of assessing pain and then, to refine experiments, we should provide analgesia.

## **Methods**

An intelligent monitoring system was developed to accurately gauge the welfare status of zebrafish after fin clipping, PIT tagging, muscle damage, skin swabbing and subcutaneous injection of acetic acid, a standard pain test. The behavioural responses to these treatments were assessed using the Fish Behaviour Index (FBI) to ascertain the state of the fish. Using this monitoring tool, a range of drugs with pain-relieving properties were tested to identify which drugs were effective in preventing the behavioural responses to pain.

## **Results**

The FBI was able to identify zebrafish subject to painful procedures and those that were not providing data demonstrating that fish in pain exhibited a profound decline in space use and activity. Certain drugs (flunixin, lidocaine and morphine) administered via immersion in the tank water were successful in preventing these responses. Skin swabbed zebrafish did not exhibit signs of pain irrespective of whether they were administered with lidocaine or not.

## **Conclusion**

This study confirms that behavioural monitoring is successful in identifying pain in zebrafish and that pain can be reduced by the use of pain-relieving drugs. This finding represents an important refinement in the use of laboratory zebrafish.

# **Non-aversive mouse handling at Lund University and the use of cage ladders**

Invited speakers

**Matilda Karlsson**<sup>1</sup>

<sup>1</sup> Rebecca Sandgren

## **Introduction**

Stress free handling is important when using laboratory animals. At Lund University we only use non-aversive handling methods for mice according to the work of Jane Hurst. This presentation will cover how we accomplished this, and what challenges we faced. It will also present the results on our study on cage ladders, that we use as cage enrichment and non-aversive handling device similar to tunnels.

## **Methods**

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## **Results**

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## **Conclusion**

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# Optimizing zebrafish rearing - effects of environmental enrichment and fish density

Invited speakers

**Oly Sen Sarma**<sup>1</sup>, *Natalia Frymus*<sup>1</sup>, *Fredrik Axling*<sup>2</sup>, *Per-Ove Thörnqvist*<sup>1</sup>, *Erika Roman*<sup>3, 4</sup>, *Svante Winberg*<sup>1</sup>

<sup>1</sup> Department of Medical Cell Biology, Uppsala University, Sweden

<sup>2</sup> Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

<sup>3</sup> Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>4</sup> Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

## Introduction

Despite its popularity in research, there is very little scientifically validated knowledge about the best practices on zebrafish (*Danio rerio*) husbandry, which has led to several facilities having their own husbandry protocols. The aim of this study was to contribute scientific knowledge on the effects of enrichment and fish density on the welfare of zebrafish in research.

## Methods

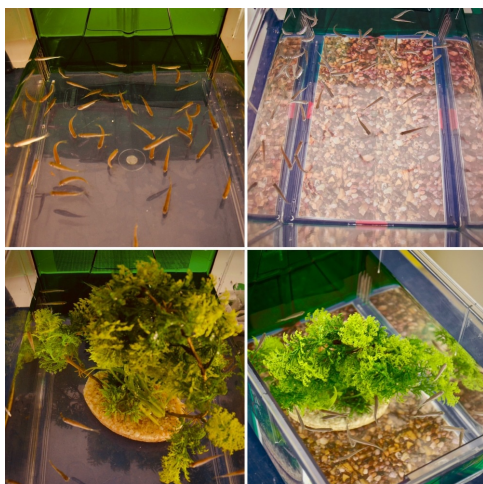
Zebrafish were reared with or without environmental enrichment at three different stocking densities, (1, 3 or 6 fish/L). Agonistic behaviour was observed twice a week for nine weeks. Cortisol secretion in response to group level stressing was analysed for each group along with cortisol secretion in response to confinement and risk-taking behaviour was assessed for individual fish. At termination, the brain tissue was sampled for analysis of brain monoaminergic activity.

## Results

Fish kept at the lowest density (1 fish/L) showed a significantly higher level of aggression and had significantly higher cortisol secretion than fish kept at the higher stocking densities when subjected to group stress. Also, these fish showed a lower dopaminergic activity than fish kept at higher densities. Fish kept at high densities showed lower and more variable growth rates than fish kept at 1 fish/L.

## Conclusion

This study shows zebrafish should not be kept at a density of 1 fish/L and the optimal stocking density will most likely to be between of 3-5 fish/L. The overall results of this study can be used to define scientifically based regulations for zebrafish housing.



## Plan your breeding to avoid producing an excess of animals

Invited speakers

**Anne Edenro**<sup>1</sup>

<sup>1</sup> Göteborgs universitet

### **Introduction**

How often do you hear that “we need to keep this mouse strain, just in case”?

This can go on for years and scientists often believe that it is efficient to keep mice in breeding, rather than cryopreserved and ready for quick expansion.

### **Methods**

We have monitored the mouse strains and colonies that are kept on maintenance breeding in our vivarium facility. Results from our investigation show that many colonies are kept over a period of several years without being used in studies.

### **Results**

This is a large problem from a 3R perspective and improvements in this area could potentially have a large impact on Reducing the number of animals used as well as Refining methods and procedures. The excessive maintenance breeding also has a negative impact on the well-being of the people who take care of the animals, as they experience that many animals are never used in research. Finally, improved breeding strategies would also free up housing capacity, allowing for more research projects.

### **Conclusion**

I will discuss timelines, cost and 3Rs in this perspective and how we at the University of Gothenburg have established and implemented a Breeding Policy to help scientist in our facility to breed smarter, more cost-effectively and how we support them in decision-making around cryopreservation. I will also discuss how we as customers can influence the vendor’s breeding strategies. As long as we keep demanding availability of large cohorts of animals, at short notice, the vendors will have to produce a large excess of animals.

# Rabbit Human Habituation Program During Breeding Reduced Significantly Stress Related Signs During Acclimatization Period

Invited speakers

**Kévin P. Dhondt**<sup>1</sup>, *Benjamin Rabany*<sup>1</sup>, *Anaïs Leal*<sup>1</sup>, *Lison Crouillé*<sup>2</sup>, *Grégory Paillet*<sup>3</sup>

<sup>1</sup> Charles River Laboratories - RMS France - Veterinary and Professional Services

<sup>2</sup> Charles River Laboratories - Safety Assessment France

<sup>3</sup> Charles River Laboratories - RMS France - Rabbit breeding

## Introduction

Rabbits are a species naturally very sensitive to stress. This stress is a source of complications for the work with these animals in laboratory settings, both in their relations with humans and on the quality of the scientific results of the research. To reduce this stress and increase animal welfare, we designed a rabbit human habituation program during breeding period, from birth to transport into experimental facility.

## Methods

The program consists in a holistic approach for positive human-rabbit bonding. It starts from birth with an impregnation program of human contact in the nestboxes 4 times/week for 4 weeks. From weaning, the proper habituation program begins with weekly positive interaction of individual petting for 2 weeks. Most of the rabbits are sold from this age. If not sold, any manipulation will be followed by a petting session. Females that are kept longer entered back into the regular habituation program from week 12 until sold with weekly positive interaction.

## Results

The effect of this program was monitored with several clinical stress indicators observed during acclimatization period. The study was designed as a double-blinded randomized study. The rabbits evaluated came for 4 different breeding areas with the habituation program implemented only in one area. Evaluators were blind on which area the program was applied.

## Conclusion

After 16 months and more than 2400 rabbits evaluated, results showed a significant decrease in stress scores of rabbits sourced from the habituation area with total disappearance of aggressive behaviors such as biting while initial scores were maintained from other areas.

# Refined methods for picking up rodents - handling change in practice

Invited speakers

**Jane Hurst**<sup>1</sup>

<sup>1</sup> University of Liverpool

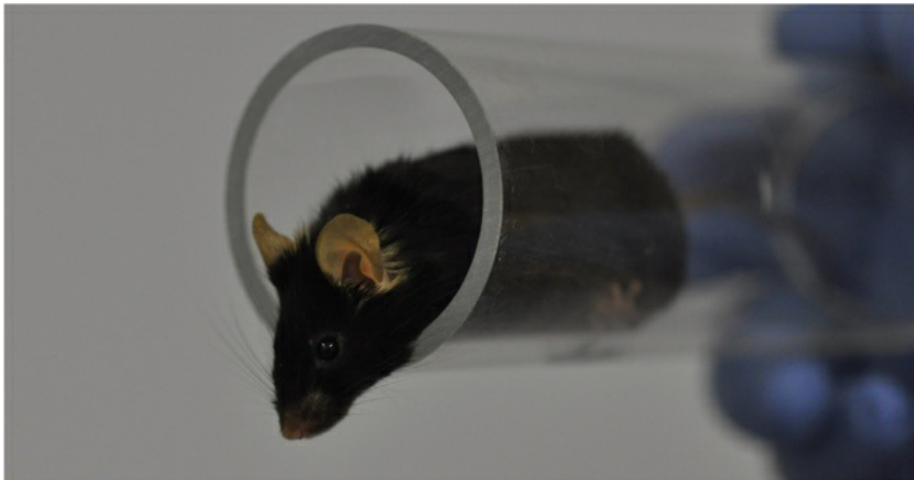
## Introduction

Routine handling of animals in the laboratory is an essential but frequently ignored component of animal experiments that has considerable potential to influence anxiety and aversion to human approach and contact. Wild-caught rodents require very careful approaches to minimise fear in captivity so that we can study their natural behaviour. Perhaps more surprisingly, domesticated laboratory mice are also highly sensitive to how they are approached and picked up. The traditional tail method consistently induces aversion and high anxiety (even with weight supported), whereas use of handling tunnels, scooping mice up on the open hand (cupping) or on a cage ladder leads to voluntary approach of the handler, low anxiety and acceptance of physical restraint. In addition to improved welfare, mice show improved performance in behavioural tests, reduced stress hormone levels, improved glucose tolerance, and improved responsiveness to sucrose rewards during experimental testing. With substantial evidence that using these less aversive methods provides a major refinement over tail handling for laboratory mice, uptake is now spreading worldwide. However, the time and effort required to achieve this has been far greater than I ever imagined. In this talk, I will summarise the current evidence base for different handling methods, share my experience of some major barriers that have slowed the uptake of this handling refinement within animal facilities, and share some tips for gaining good technique and successful implementation.

## Methods

## Results

## Conclusion



## Rodent anesthesia: optimization, refinement, and monitoring

Invited speakers

**Moustapha Hassan**<sup>1, 2</sup>, *Daniel Bergman*<sup>1, 2</sup>, *Ying Zhao*<sup>1, 2</sup>

<sup>1</sup> Pre-Clinical Laboratory, Karolinska University Hospital, Huddinge

<sup>2</sup> Department of Laboratory Medicine, Karolinska Institutet

### Introduction

Anesthesia is a key issue in preclinical studies using laboratory rodents. A poorly designed anesthesia protocol will lead to wasteful use of animals due to morbidity and mortality, and a higher experimental inter- and intra-individual variation. Proper anesthesia can improve the quality of preclinical studies, improve animal welfare, and decrease animal usage/cost.

### Methods

Anesthesia was originally developed for human use; knowledge of rodent anesthesia is mostly derived from human beings as large animals with the similar mechanism across species. However, it is more challenging to refine rodent anesthetic procedures due to the smaller size and faster metabolism in rats and mice. To achieve the optimal anesthesia outcome, the right depth and duration of anesthesia suitable for each individual study, it is necessary for researchers and veterinarians to collaborate to: 1) Refine the anesthesia protocols using optimal administration routes and dosing. 2) Conduct adequate anesthesia and perioperative physiological monitoring; 3) Prevent anesthesia-induced complications by perioperative administration of multimodal analgesia.

Moreover, in longitudinal studies using non-invasive in vivo imaging, anesthesia is applied repeatedly at different time points to restrain the animals and reduce artifacts from movement.

### Results

Both anesthetic agents and imaging procedures may affect the physiological status of the animal and consequently the imaging acquisition. Therefore, real-time physiological monitoring systems are implemented during imaging sessions. It is also worth noting that there are potential side effects from anesthetic agents, *e.g.* depression of respiration, cardiovascular function and thermoregulation.

### Conclusion

Good anesthesia protocols will positively affect the experimental outcome as well as the animal welfare.

# STAY OUT OF MY TERRITORY - understanding and mitigating male mouse aggression

Invited speakers

**Tamara Baker**<sup>1</sup>, *Sofia Ostman*<sup>2</sup>, *Birgit Edwaldsson*<sup>2</sup>, *Sally Robinson*<sup>3</sup>, *Kate Shenton*<sup>3</sup>, *Robbie McLaren - Jones*<sup>1</sup>, *Dawn Atherton - Kemp*<sup>1</sup>, *Therese Edstrom*<sup>2</sup>, *Amir Hussain*<sup>4</sup>, *Sara Albery Larsdotter*<sup>2</sup>, *Amy Cantrell*<sup>3</sup>, *Natalie Kelley*<sup>5</sup>, *Diana Pao*<sup>5</sup>

<sup>1</sup> AstraZeneca, Cambridge, United Kingdom

<sup>2</sup> AstraZeneca, Gothenburg, Sweden

<sup>3</sup> AstraZeneca, Macclesfield, United Kingdom

<sup>4</sup> AstraZeneca, Gaithersburg, United States

<sup>5</sup> AstraZeneca, Boston, United States

## Introduction

Group housing is very important for social animals. However, it can also give rise to aggression, (particularly within the laboratory environment) which is one of the most serious welfare concerns in mouse husbandry. Severe fighting can lead to pain, injury and death.

Historically male mice have been treated the same as female mice when handling and during husbandry procedures. Understanding the triggers of aggression has led to us implementing a specific male mouse housing and handling regime.

Sex bias is a major issue in the pre-clinical setting, we cannot simply not use male mice in studies. Additionally male mice are required for certain models e.g. prostate cancer models. When using male mice often additional mice have to be included to mitigate the potential loss of mice due to fighting and keep the study statistically relevant.

## Methods

We formed a global working group to identify the triggers of aggression e.g. behaviours seen prior to fighting occurring and have identified strategies to mitigate these triggers. We have also identified the signs of a harmonious cage, how to identify when aggression is occurring and when/ how to intervene.

## Results

Utilising these new regimes has greatly reduced overt aggression and ensured more harmonious social interactions. This has led to a reduction in the number of animals that needed to be separated from 31% to less than 1% in nude mice.

## Conclusion

Understanding what triggers aggression and the more subtle signs of aggression has allowed us to take action before animals need to be euthanised.

# Swedish presidency; What happens in the EU related to the animal welfare legislation?

Invited speakers

**Helena Elofsson**<sup>1</sup>, *Cecilia Bornestaf*<sup>1</sup>

<sup>1</sup> Swedish Board of Agriculture

## **Introduction**

During the first six months of 2023, Sweden is the President of the Council of the European Union.

Sweden will during this time address several important issues in the animal and veterinary field, for example issues related to the ongoing review of the EU-Animal welfare legislation.

During this presentation, we will give an overview of the ongoing work with these issues.

We will also give a brief update of the ongoing EU Commission work to amend Annex III (Care & Accommodation Requirements) and Annex IV (Killing Methods) of Directive 2010/63/EU,

the EU Citizens' Initiative *Save cruelty free cosmetics - commit to a Europe without animal testing* as well as new features and e-learning modules on the Education and Training Platform for Laboratory Animal Science, etplas.eu.

## **Methods**

## **Results**

## **Conclusion**

# the legal use of biocides within a biomedical facility under the EU biocidal products regulations (BPR)

Invited speakers

**John Edwards**<sup>1</sup>

<sup>1</sup> Ecolab UK Ltd

## **Introduction**

A quick look at the BPR and its implications for those biomedical facilities using automated airborne disinfection systems and chemistry for biocidal use. Explaining what approvals are required to allow the legal application of a biocide in a biomedical facility. The presentation will also cover a method of quickly checking the BPR approval status of any given supplier or chemistry as well as highlighting the enforcement agencies within Scandinavia.

## **Methods**

Power point presentation given verbally

## **Results**

Hopefully positive

## **Conclusion**

Audience should have a basic understanding of the BPR, its legal implications and where to go to check on compliance.





## **Their care is in our hands**

Invited speakers

**Amelie Scholtz**<sup>1</sup>

<sup>1</sup> Envigo

### **Introduction**

Have you ever thought about what happens in the early life of your animals at a commercial vendor? Do you know what the common practices of the vendor are? What are your animals used to concerning housing, enrichments, diet and bedding, but also handling? And what else is done to take good care of your animals?

During this presentation, you will learn the common practices of Envigo for our rodents within Europe, including the non-aversive handling techniques that are used. Besides taking good care of our animals and colleagues, we believe that the culture of care goes beyond the boundaries of our company. Through participation in the EARA transparency agreement and by supporting our customers, we believe that we are truly taking good care of our animals.

### **Methods**

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### **Results**

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### **Conclusion**

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## Training of pigs in experimental studies

Invited speakers

**Anneli Ryden**<sup>1</sup>

<sup>1</sup> University of Agricultural Sciences, Uppsala, Sweden

### **Introduction**

At the faculty of Veterinary medicine and animal science, SLU, Uppsala, experimental studies are carried out on pigs both to study function and diseases of the animal species and the outcome of surgical interventions in collaboration with medical expertise. The 3Rs, must be considered when working with laboratory animals. Refinement in nursing and handling are desirable for improving the welfare of pigs in our faculty. Research on untrained pigs may induce stress and discomfort, therefore studies on pigs concerning stress-free handling, and appropriate handling techniques regarding the perioperative period are required.

### **Methods**

The pigs arrives at least two weeks before start of the study, and the specific training programme starts for the individual animal and considering the specific design of the study. All technicians, nurses, veterinarians and co researchers are involved from planning until the end of the study.

### **Results**

This results in calm animals, which allows for examinations, blood- and urine sampling etc. without stress for the animals or for the involved staff taking care of the animals

### **Conclusion**

The method is successful at our faculty and can be applied into the everyday work with the animals and contributes to animal welfare, care of staff, transparency, and scientific quality.

## **Use of scoring sheets for determination of human endpoints**

Invited speakers

**Maria Averstad** , *Kristian Konigsson*

### **Introduction**

Use of scoring sheets for determination of human endpoints:

A workshop describing a way to teach and use a template for assessing clinical signs of disease and suffering in laboratory animals. At Karolinska Institutet, an assessment template has been used for many years to describe humane endpoint in animal experiments. The use of the template requires training for both researchers and staff. For researchers training is required - since they need to interpret clinical signs of their animals and define accurate humane endpoints for their experiments in order to achieve their scientific goals without causing unnecessary suffering to the animals. For the technical staff training is needed for usage of the template in a consistent way, and to make sure that the technicians and veterinarians are calibrated and have a uniform view of clinical signs, their interpretations and make similar assessments. Templates with well-defined clinical signs can also be used for refinement since they could provide measurable parameters possible to use for benchmarking.

### **Methods**

### **Results**

### **Conclusion**

# **Working with non-human primates: a challenge that requires daily commitment, patience and resourcefulness.**

Invited speakers

**Mikaela Sandbacka**<sup>1</sup>

<sup>1</sup> KMF/AFL KM, Karolinska Institutet

## **Introduction**

At the Astrid Fagraeus Laboratory, at the Karolinska institutet, Sweden, we keep many different species of laboratory animals in biosecurity level 1-3. As the only non-human primate (NHP) facility in Scandinavia, 2 species of NHP are housed, currently 55 crab-eating and rhesus macaques. This presentation will show how we work with our NHPs on daily basis, with a focus on enrichment and training. Housing NHPs includes a lot of challenges and requirements from different points of view, legal, scientific and species-specific demands. In the light of this, staff working with NHPs must be very experienced, committed and hardworking to keep primates in good mental and physical health and primate enclosures clean, safe and with an intact 3-dimensional interior design. Working with NHP requires patience and passion and our NHPs are amazing and make every work day interesting and exciting.

## **Methods**

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## **Results**

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## **Conclusion**

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## Workshop on choosing the right experimental design

Invited speakers

**Simon Bate**<sup>1</sup>

<sup>1</sup> CMC Statistics, GlaxoSmithKline Medicines Research Centre, Stevenage, UK

### **Introduction**

In this workshop, we shall consider how the purpose of an experiment influences the choice of experimental design. Attendees will explore three stages of experimentation, namely: pilot studies; hypothesis-generating experiments; and hypothesis-confirming experiments.

### **Methods**

By working through a practical example, in an interactive session, we shall consider three key questions: 1) what is the purpose of the experiment at each stage of the study; 2) how does the purpose of an experiment affect its design; and 3) what conclusions can be drawn from different types of statistical analysis?

### **Results**

Results will be generated, and assessed, during the workshop in real time.

### **Conclusion**

A key theme of the workshop will be to consider how to reduce animal use, while maintaining the scientific validity and reproducibility of conclusions drawn from the study.