



Abstracts of scientific presentations Scand-LAS 2023 symposium Uppsala, Sweden

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Lectures and Oral communications

Karl Johan Öbrink Memorial Lecture (Keynote)

Refined methods for picking up rodents – handling change in practice

Jane Hurst

University of Liverpool, UK

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 to 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 hand-

ling 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.

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

Lotte Martoft

Aim2Achieve

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 at 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.

Assessing animal welfare – the familiar and the ‘exotic’

Oliver Burman

University of Lincoln, UK

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. 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. 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. 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.

A standardised score sheet template for assessing rodent health

Elin Spangenberg¹, Patricia Hedenqvist¹, Emelie Jansson², Erika Roman^{3,4,5}

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Score sheets formalise and standardise the assessment of welfare and make it possible to record the impacts of scientific procedures. The Swedish National Com-

mittee 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. 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. The template can be used to assess animals’ health before, during and after a study to estimate if health has deteriorated, as well as to decide if the humane endpoint is reached. It can also 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. Our template has been shared nationally and several facilities have started to use it to assess the health of their rodents.

Session: Training of animals with emphasis on Culture of Care

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

Mikaela Sandbacka

Karolinska Institute, Sweden

At the Astrid Fagraeus Laboratory, at the Karolinska Institute, 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 a 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 workday interesting and exciting.

Training of pigs in experimental studies

Anneli Ryden

University of Agricultural Sciences, Sweden

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 using stress-free handling of pigs, and appropriate handling techniques during the perioperative period, are required. The pigs arrive at least two weeks before the start of the study, and the specific training programme starts for the individual animal taking into consideration the specific design of the study. All technicians, nurses, veterinarians and co-researchers are involved from planning until the end of the study. This results in calm animals, which allows for examinations, blood- and urine sampling etc. without stress for the animals or for the staff taking care of the animals. 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.

Socialization of pigs and rabbits - preparation for training

Stine Drent Larsen, Sarah Ladefoged Sharpe

Novo Nordisk A/S, Denmark

As animal caretakers, we want to work with animals in the best possible way, for both caretaker 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 animals during the acclimation period by incorporating socialization, habituation and training. As the animals are participating in studies of 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. We want to share our approach to socialization and 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. We will also touch upon socialization and enrichment initiatives in our rabbit facility, e.g. marking, housing and handling. We will also give examples of standard training and more complex study specific training and demonstrate this with pictures and videos. At Novo Nordisk we socialize our animals to improve their welfare, with this approach comes reduced stress, potentially more reliable data and more accurate dosing, easier cooperation and calmer animals.

Session: Communication

Animal Research: Time to Talk!

Gege Lie

European Animal Research Association

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, i.e. in 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 animals 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.

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

Jenny Berrio

University of Copenhagen, Denmark

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

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

Svea Jörgensen, Elin Weber, Johan Lindsjö, Frida Lundmark Hedman, Helena Röcklinsberg
Swedish University of Agricultural Sciences, Sweden

Directive 2010/63/EU states that a “climate of care” should be cultivated when breeding, keeping and using research animals and that “animal-welfare considerations should be given the highest priority”. It has been in place for more than a decade and thus a relevant question to ask is how well Member States are living up to this goal. Consideration, application and fulfilment of the 3Rs from project-planning to retrospective review are vital aspects 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 for ethical review. 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 often confused with, and described as, one

another by researchers. 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 applications can the animals’ situation be fully understood, and competent authorities ensure that projects are only approved where the benefits outweigh the harms.

Session: Culture of Care and 3Rs in practice – how to make a change

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

Marie Hornfelt

University of Gothenburg, Sweden

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 research staff. 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 community, 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 scientists 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, what we have learnt as well as some of the challenges we are facing.

Learning from failure – fostering the Culture of Care

Sofia Östman

AstraZeneca, Sweden

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 is also an essential component of securing sustained and improved animal welfare. Talking openly about what goes wrong can be a challenge for staff as it entails sharing examples and situations of “near misses” or “failures”. To work effectively with learning 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. 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 learn from errors effectively. The log is an automated process that includes an easily accessible event reporting tool called “Learning Log” and Root Cause Analysis. I will discuss our approach (pros, cons, requirements) and provide advice on how to address this new way of working in trustful collaboration with staff.

Cage change on demand, challenges with implementation

Rebecca Sandgren

Lund University, Sweden

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 affects 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 affecting 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.

Implementing cup and tunnel handling in a pharmaceutical rodent facility

Maria Kiersgaard, Marie Petersen, Peter Lund Gade, Helle Nordahl Hansen

Novo Nordisk A/S, Denmark

The way of handling mice has been by catching the animal by the tail, lifting it and generally holding the mouse by the tail; this was considered the only effective and safe way of handling. In 2010 the first article on cup and tunnel handling was published. 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. (Taming anxiety in laboratory mice. Hurst, J. and West, R. Nat. Methods 7, 825–826 doi:10.1038/nmeth.1500, 2010). In 2015, we began to look into cup and tunnel handling, however, without the correct instructions to staff 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 working.
- 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 animal caretakers. 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.

Culture of Care – what does it look like at Novo Nordisk

Cathrine Juel Bundgaard, Johan Mikkelsen

Novo Nordisk A/S, Denmark

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 establishments working with laboratory animals have implemented or developed their own and unique animal welfare and the 3Rs culture. 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 then choose solutions which best match 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 and show empathy with the animals. 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. 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.

Insight – Practical implementation of laboratory animal monitoring and veterinary care

*Søren Søgaard, Marianne Ravn Møller
Gubra, Denmark*

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 wellbeing of the animals and highlight Culture of Care. Our animal care program now consists of cooperation between study directors, animal technicians and laboratory animal veterinarians to ensure an efficient daily monitoring of the animals. Monitoring comprises both objective parameters (e.g., body weight or food intake), and clinical parameters based on daily visual inspections by animal caretakers and veterinarians. Together with our internal computational biology team, we have developed a weight monitoring system to immediately upload data to our internal data explorer. This gives study directors and veterinarians an excellent tool for monitoring the animals and ensure that animals without clinical or external signs of disease will be assessed daily based on differences in objective parameters. Animal caretakers are instructed to look for a variety of parameters when handling the animals. If veterinary attention is required, the observations are passed via a designated email 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 become part of the daily schedule. These measures have led

to a rapid response assuring that animals receive the proper treatment and care they need.

Session: Novel approaches

Home cage monitoring of mice

Vootele Voikar

University of Helsinki, Finland

Mice are the most commonly used laboratory animals in biomedical research. Assessment of behavioural 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 animal welfare relies mostly on cage-side observations. In both situations, the duration of observation is usually very short and occurs in most facilities during the light phase of the circadian cycle, thus rendering a high possibility for missing subtle changes in behaviour. Moreover, behavioural 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 behavioural and physiological parameters of animals in their home cages. In 2021, a pan-European network of researchers started a 4-year COST (European Cooperation in Science and Technology) Action “Improving biomedical research by automated behaviour monitoring in the animal home-cage” (CA20135 TEATIME). For this project, experts from different fields collaborated to critically assess the potential of available technologies, to develop guidelines and identify the needs for further technological development, including analysis of big data. A 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-tea-time.org/>.

Behavioural profiling of rats, mice and zebrafish

Erika Roman

Swedish University of Agricultural Sciences, Sweden

Increased understanding of complex behaviours may require new approaches. Conventional behavioural tests offer limited possibilities to capture a broad behavioural repertoire, and if combined into test batteries there is a risk of carry-over effects. The multivariate concentric square field™ (MCSF) test, originally developed for rats, is unique in its design by provoking behaviours associated with exploration, risk taking and shelter seeking. Thereby a behavioural profile is generated in a single session. In this presentation, examples of the use of the MCSF test for behavioural profiling of rats, mice and zebrafish will be presented. The MCSF test may constitute an important complement to conventional tests used in preclinical research.

Modelling the child eye cancer retinoblastoma using chicken embryonic retina

Finn Hallböök, Dardan Konjusha, Minas Hellsand, Hanzhao Zhang, Maria Blixt, Henrik Ring
Uppsala University, Sweden

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. 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. MYCN transformed chick retinas developed tumours at high frequency, expressing a cone photoreceptor progenitor phenotype that was resistant to apoptosis in contrast to other cell types. Cells proliferated beyond the normal post-mitotic states and formed metastatic cancer that infiltrated the 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. 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-rich cone-poor retina is less good. The augmented E2F signalling in MYCN-driven retinoblastoma was identified as a potential new drug target.

Session: Culture of Care at the vendors

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

Giorgio Rosati

Tecniplast SpA, Italy

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. 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.

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

- The examination of polyuria as a marker of sustained hyperglycaemia 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

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.

Session: The 3Rs in education

Assuring competence in practical tasks – ETPLAS working group 4

Lucy Whitfield¹, Andreia Costa², Eirini Fragiadaki³, Rafael Frias⁴, Brian Mphande⁵, Paul West⁶

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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, 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 staff 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.

ETPLAS working group developed a 'library' of DOPS (Directly Observed Practical Skills) for assessment of commonly used practical techniques. DOPS have the following features:

- 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
- Competence criteria should be:
- Relevant to the task
 - Easy to measure
 - Available to the trainer, trainee & assessor so everyone is clear about the standard required for competence.

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

Adrian Smith

Norecopa, Norway

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. Norecopa's staff have been collecting information about alternatives and supplements to animal use in education since 1991. This includes 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. 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 become available, and old ones go out of production. NORINA currently 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. This presentation will show case examples of a wide range of resources which are relevant to the field of laboratory animal science.

Session: Culture of Care in research on wild animals

Culture of Care in the wild - does anyone care?

Jonas Malmsten

Swedish University of Agricultural Sciences, Sweden

Research on 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 several ways of which some are highly questionable. Except for 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 is 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. 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.

Session: Male mouse aggression

Stay out of my territory - understanding and mitigating male mouse aggression

Tamara Baker¹, Sofia Ostman², Birgit Edwaldsson², Sally Robinson³, Kate Shenton³, Robbie McLaren-Jones¹, Dawn Atherton-Kemp¹, Therese Edstrom², Amir Hussain⁴, Sara Albery Larsdotter², Amy Cantrell³, Natalie Kelley⁵, Diana Pao⁵

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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 must be included to mitigate the potential loss of mice due to fighting and keep the study statistically relevant. We formed a global working group to identify the triggers of aggression

e.g. behaviours seen prior to fighting 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. 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. Understanding what triggers aggression and the more subtle signs of aggression has allowed us to take action before animals need to be euthanised.

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

Elin M Weber¹, Charlotte Berg¹, Joseph P Garner²

¹University of Agricultural Sciences, Sweden, ²Stanford University, California, USA

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 behaviours and fighting). Behavioural 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. We will discuss three studies. First, the largest and most comprehensive study of any behavioural 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. Informed by this work, we are currently conducting

a full-scale epidemiological study including several Swedish research facilities.

Session: Pain management

Monitoring pain in zebrafish

Lynne Sneddon

University of Gothenburg, Sweden

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. 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. The FBI was able to identify zebrafish subject to painful procedures and those that were not; 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. 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.

Best anaesthesia protocol for fish species and situation

Albin Gräns

Swedish University of Agricultural Sciences, Sweden

In this project, we determined how different anaesthetics affect the health and welfare of fish and to what extent the effects are context dependent. We aimed to determine the best anaesthetics for three

model species (i.e. zebrafish, brown trout and rainbow trout), but the results will be valid for a range of fish species. Here we conducted a series of studies covering the induction time of unconsciousness, different drugs, to the consequences for animals being released back into their home environments. We used a range of state-of-the-art methods; including non-invasive techniques 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. This is the first comprehensive evaluation of various common anaesthetic compounds based on monitoring 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 variation both between and among species. Results and conclusions will be presented and discussed in relation to our three model species.

Session: Pain management

Effective pain management in laboratory animals

Klas Abelson¹, Carsten Grøndahl²

¹University of Copenhagen, ²Copenhagen Zoo, Denmark

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 results. 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, particularly in rodents, is difficult and time consuming and thus the validation of an effective pain treatment is challenging. The risk of skewing experimental data due to potential confounding effects of the analgesic drugs must also be taken into consideration and eliminated. This session will give an introduction to pain and nociception in animals and presents and discusses different strategies for 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.

Session: The 3Rs in education

Animal technicians: The key persons for practical skills and competences

Klas Abelson, Åsa Holmberg, Siri Knudsen, Lene Gorm Pedersen, Toomas Tiirats, Hanna-Marja Voipio
Scand-LAS Education and Training Committee

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. 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, whereby 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. 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. 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!

Session: Refinement

Designing your own cardboard mouse house

Jenny Bendtsen
University of Gothenburg, Sweden

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 mouse enrichment was to provide addition-

al 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 empty toilet rolls and glove boxes, with an undefined chemical content and required a labour-intensive handling process. As we found commercially available mouse hideouts (i.e. houses or tunnels) cost-ineffective, we developed 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. Our cardboard mouse house is cheaper than commercially available hideouts and is made of material completely free from plastic, glue, dyes or other added chemicals.

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

Matilda Karlsson
Lund University, Sweden

Stress free handling is important when using laboratory animals. At Lund University we only use non-aversive handling methods for mice, in accordance with 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, which we use as cage enrichment, and non-aversive handling device similar to tunnels.

Optimizing zebrafish rearing – effects of environmental enrichment and fish density

Oly Sen Sarma¹, Natalia Frymus¹, Fredrik Axling¹, Per-Ove Thörnqvist¹, Erika Roman,^{1,2} Svante Winberg¹

¹Uppsala University, ²Swedish University of Agricultural Sciences, Sweden

Despite its popularity in research, there is very little scientifically validated knowledge about the best practices for 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. Zebrafish were reared with or without environmental enrichment at three different stocking densities, (1, 3 or 6 fish/L). Agonistic behaviour was monitored 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. 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. This study shows zebrafish should not be kept at a density of 1 fish/L and the optimal stocking density will be most likely to be between of 3–5 fish/L. The overall results of this study can be used to develop scientifically based regulations for zebrafish housing.

Session: Monitoring animals under anaesthesia

Rodent anaesthesia: optimization, refinement, and monitoring

Moustapha Hassan, Daniel Bergman, Ying Zhao
Karolinska University Hospital, Karolinska Institute, Sweden

Anaesthesia is a key issue in preclinical studies using laboratory rodents. A poorly designed anaesthesia protocol will lead to wasteful use of animals due to morbidity and mortality, and greater experimental inter- and intra-individual variation. Proper anaesthesia can improve the quality of preclinical studies, improve animal welfare, and decrease animal usage/cost. Anaesthesia was originally developed for human use; knowledge of rodent anaesthesia is mostly derived from humans. However, it is more challenging to refine rodent anaesthetic procedures due to the smaller size and faster metabolism in rats and mice. To achieve the optimal anaesthesia outcome, the right depth and duration of anaesthesia suitable for each individual study, it is necessary for researchers and veterinarians to collaborate to: 1) Refine the anaesthesia protocols using optimal administration

routes and dosing, 2) Conduct adequate anaesthesia and perioperative physiological monitoring, 3) Prevent anaesthesia-induced complications by perioperative administration of multimodal analgesia. Moreover, in longitudinal studies using non-invasive in vivo imaging, anaesthesia is applied repeatedly at different time points to restrain the animals and reduce artefacts from movement. Both anaesthetic 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 anaesthetic agents, e.g. depression of respiration, cardiovascular function and thermoregulation. Good anaesthesia protocols will positively affect the experimental outcome as well as animal welfare.

Monitoring animals under anaesthesia in the wild

Åsa Fahlman
SLU Swedish Biodiversity Centre, Sweden

Capture and anaesthesia 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 anaesthesia. Thus, evaluation and monitoring of anaesthesia 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 anaesthesia, oxygen therapy is essential and 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, anaesthesia and monitoring, as well as prevention and treatment of complications.

Monitoring long-term anaesthesia in pigs

Miklos Lipcsey
Uppsala University, Sweden

Anesthetizing animals for hours to days poses challenges for maintaining hemostasis. The aims of anaesthesia are to provide optimal conditions for experimental research without causing animal suf-

fering by achieving loss of consciousness, analgesia, inhibition of autonomic reflexes, immobilization, and in non-terminal experiments amnesia. Anaesthesia 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; this requires specific knowledge about the physiology of the laboratory animals as well as the pharmacology of the used substances. Clinical monitoring by being continuously attended by staff who can identify signs of stress or deterioration in vital functions 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 and body temperature.

Session: Reduction

Establishment of a searchable database for sharing tissues from experimental animals

Leif Carlsson

Umeå University, Sweden

The concept of the 3Rs (reduce, refine and replace) has to be considered by all scientists using animals in their research. An important contribution to the 3Rs would be if scientists 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. 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 details of their samples (tissue type, strain, sex, genotype etc), and search for other tissues for their own research. This concept aims to show the viability of implementing a common animal tissue database that could be available for the scientific community. Such a database would contribute to a reduction of the number of animals used in research, as well as boosting collaboration between institutions and countries.

Plan your breeding to avoid producing an excess of animals

Anne Edenro

Göteborg University, Sweden

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 on maintenance breeding, rather than cryopreserved, and ready for quick expansion. 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. This is a significant 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. 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. I will discuss timelines, cost and 3Rs from this perspective and how we at the University of Gothenburg have established and implemented a mouse Breeding Policy to help scientists in our facility achieve smarter and more cost-effective use of animals, and how we support them in decision-making around cryopreservation. I will also discuss how we as customers can influence the vendor’s mouse breeding strategies. As long as we keep demanding availability of large cohorts of animals, at short notice, vendors will have to produce a large excess of animals.

Session: Culture of Care at the vendors

Their care is in our hands

Amelie Scholtz

Envigo

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 (European Animal Research Association) transparency agreement and by supporting our customers, we believe that we are truly taking good care of our animals.

Rabbit-human habituation program during breeding reduced significantly stress related signs during acclimatization period

*Kévin P. Dhondt, Benjamin Rabany, Anaïs Leal, Lison Crouillé, Grégory Paillet
Charles River Laboratories*

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 the breeding period, from birth to transport into an experimental facility. The program consists in a holistic approach for positive human-rabbit bonding. It starts from birth with a program of human contact in the nest-boxes 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 at this age. If not sold, any manipulation will be followed by a petting session. Females that are kept longer are entered back into the regular habituation program from week 12 until sold with weekly positive interaction. The effect of this program was monitored with several clinical stress indicators observed during the acclimatization period. The study was designed as a double-blinded randomized study. The rabbits evaluated came from 4 different breeding areas with the habituation program implemented only in one area. Evaluators were blind on which area habituation was applied. After 16 months and more than 2400 rabbits evaluated, results showed a significant decrease in stress scores for rabbits sourced from the habituation area with total disappearance of aggressive behaviours such as biting while initial scores were maintained from other areas.

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

*Maja Ramløse, Kirsten Rosenmay Jacobsen, Carina Anker, Susi Søgaard
Ellegaard Göttingen Minipigs A/S, Denmark*

Creating and maintaining a good Culture of Care, and working with 3Rs, is a continuous task with many positive outcomes: better science, better animal welfare and better working terms for the staff. 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 of minipigs has many physiological and behavioural 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 playrooms. 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 3Rs 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.

Automated blood sampling: does it contribute to the 3Rs?

*Edwin Spoelstra, Xanne Ketelaar, Horst Beier
UNO Life Science Solutions*

Acute blood sampling techniques such as facial vein and tail vein are commonly used in mice and rats. These acute techniques require the animal to be restrained; physically (immobilization) or chemically (anaesthesia). Restraint compromises the “true” value of blood drug concentration. Freely moving blood sampling – in dwelling catheters – is the preferred method to obtain these “true” values. Automated blood sampling (ABS) in combination with freely moving animals can further contribute to obtaining “true” values of blood drug concentration. ABS also contributes significantly 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.

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

John Edwards

Ecolab UK Ltd

This talk will provide a quick look at the BPR and its implications for those biomedical facilities using automated airborne disinfection systems and chemistry for biocidal use. The approvals required to allow the legal application of a biocide in a biomedical facility will be explained. 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.

Dry heat sterilization in vivarium – an alternative option for sterilization in the lab animal facility

Robert Davis, Tine Jensen

Opend ApS, Denmark

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
- Eight 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

tant 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.

Session: Swedish EU presidency

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

Helena Elofsson, Cecilia Bornestaf

Swedish Board of Agriculture, Sweden

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 impor-