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Scientific Abstracts from the 45th Scand-LAS Annual Meeting and Educational Days

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Lectures and Oral communications

Session: Better Science

Modern cancer research – from biophysics to *in vivo* assays

Karl-Johan Öbrink Lecture (Keynote)
Johanna Ivaska
University of Turku, Finland

Cancer is a general name for more than 200 distinct diseases originating from different tissues. Cancer is a genetic disease where mutations in cells drive transformation and result in uncontrolled growth of cells giving rise to a tumour. However, during tumour progression the tumour microenvironment, including the surrounding tissue, is critical in supporting the malignant development. The host tissue plays also an essential role in the regulation of tumour dissemination and metastasis. Thus, cancer is a systemic disease involving a complex interplay between the cancer cells and the host tissue. Modern cancer research implements many techniques for investigating the behaviour of cancer cells with the ultimate aim to generate better and more effective therapies against cancer. The generation of complex *in vitro* tissue-like model systems has enabled researchers to replace the use of experimental animals for some aspects of their research. In addition, the use of model organisms like fruit flies, fish, worms and chicken eggs has enabled researchers to perform studies *in vivo* without the need to use mammals. Importantly, sophisticated imaging techniques enable researchers to develop more refined cancer models that require significantly fewer animals than was necessary in the past. Nevertheless, complicated systemic diseases like cancer need to be investigated using *in vivo* models both in the areas of basic research as well as in drug development. Alternative models are unlikely to ever fully replace the need for experimental animals in biomedical research.

Translational values of mouse models in oncology drug

Melissa Junttila
Genentech Inc., USA

During this seminar, I will provide insight into the appropriate application of *in vivo* mouse modelling of cancer to inform oncology drug development. Much debate exists about the pros and cons of various oncology model systems with the overall conclusion that these models lack the predictive power required to translate preclinical efficacy into clinical

use. Herein, I will provide a balanced, albeit critical view of these claims and outline a framework for proper use of existing preclinical models for drug testing and discovery. I will also discuss the historical evolution of target identification and how the recent focus on low frequency oncogenic mutations and immune-based therapies has altered the way we use these model systems. Lastly, I will highlight gaps in the current suite of oncology mouse models and propose ways to address them.

Animal models of symptomatic epilepsy

Xavier E. Ndode-Ekane
University of Eastern Finland, Finland

Epilepsy is one of the most common chronic neurological disorders affecting approximately 1% of the world population. By definition, it is characterised by an enduring predisposition to generate epileptic seizures and includes a history of at least two seizures occurring 24 h apart. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Symptomatic epilepsy is the most common form of epilepsy. It usually begins with an initial precipitating brain injury, followed by several tissue remodelling processes (epileptogenesis), which makes the brain prone to seizures, eventually culminating in the occurrence of spontaneous seizures. There is an enormous challenge to develop relevant experimental animal models that replicate the complex mechanisms underlying epileptogenesis and seizure generation in humans. This challenge has become even more difficult due to the growing political and ethical concerns about the use of animals in biomedical research. Nonetheless, several experimental models that mimic different aspects of the epileptic process have been developed. Symptomatic epilepsy models include the chemoconvulsive or status epilepticus models (kainate and pilocarpine models), electrical stimulation models and brain pathology models (stroke, hyperthermia, hypoxia and traumatic brain injury models). These models have help us to understand the nature of the precipitating brain injuries (stroke, trauma, tumour, status epilepticus), the different processes that contribute to the disease progression (epileptogenesis), and to study the chronically epileptic brain using various techniques. Furthermore, these models are essential for developing new treatment strategies and testing the efficacy of antiepileptic drugs.

Neuroimaging of small animals with PET tracers

Francisco R. Lopez Picon
University of Turku, Finland

Positron emission tomography (PET) is a non-invasive molecular imaging technique. PET measures the *in vivo* biodistribution of positron-emitting radionuclide labelled compounds that are used as labelling agents. The main goal of PET imaging is to characterize biological processes in tissues and organs with minimally invasive procedures. PET is well established as an important research and clinical molecular imaging technique. The marked size difference between human brain (1400 g), rat brain (2 g) and mouse brain (0.4 g), has posed a significant challenge for the use of PET imaging in small animals. The technological advances in the last decade have allowed the production of a PET scanner for small animals with higher resolution (~1 mm) than those used for humans (~4 mm). These changes have enabled the reliable use of PET imaging for preclinical applications. Imaging brain in small animals with different PET tracers is the most attractive method for detection and follow-up of pathologies and treatments because of its ability to directly visualize, map and quantitate. Furthermore, the use of PET imaging in longitudinal studies allows for a very significant reduction in the number of animals used, and a refinement of the experiments.

Session: Better Welfare

Directive 2010/63/EU on the protection of animal used for scientific purposes

Update from the EU

Susanna Louhimies
DG Environment, the European Commission, Belgium

Approximately 12 million animals are used on a yearly basis in scientific procedures in the EU. The ultimate aim of the EU is a full replacement of the use of animals in research and testing. However, the current scientific knowledge does not yet allow this goal to be achieved; animals are still needed to safeguard human and animal health and the environment. This is why EU legislation for the protection of animals used for scientific purposes was strengthened in 2010 through the adoption of Directive 2010/63/EU. It took full effect on 1 January 2013 and all Member States have transposed it into national

legislation. With the Directive, the EU can claim to have the highest standards of experimental animal welfare in the world, whilst promoting high quality, competitive science and research in Europe. However, the Directive can only deliver if its transposition is correct and complete, and when its day to day application is embraced by all those affected by the legislation. To achieve this, there needs to be a uniform understanding of the Directive's obligations as well as tools for those implementing them. Since its adoption, the Commission has engaged in a continuous dialogue with the stakeholder community to address a number of topics that were identified as benefitting from further guidance. These topics include *inter alia* the severity assessment framework, a mutually recognisable education and training framework, project evaluation/retrospective assessment, the functioning of animal welfare bodies and national committees, and inspections and enforcement.

Finally, just last week the Commission published its response to the Citizens' Initiative "Stop Vivisection!" in a formal Communication setting out its views on the protection of animals used in science, The Citizens' Initiative is a new democratic agenda-setting tool provided by the Treaty of the Functioning of the European Union.

Experimental pain models for clinical conditions

Outi Vainio
University of Helsinki, Finland

The use of laboratory animals in pain research has contributed a great deal to the understanding of pain and pain alleviation in humans. However, recent studies have shown that preclinical animal pain models have repeatedly failed to predict clinical analgesic efficacy of novel drug molecules aimed for use in human pain patients. This paper presents methods for observing acute and chronic pain in animal patients who are presented to a veterinary clinic. Both subjective and objective tools are available. Subjective information on the quality of life of pets suffering from pain can be collected by validated questionnaires. Gait analysis of veterinary pain patients will give objective information on the severity of limping which is considered as a sign of pain. New imaging techniques to detect painful body areas have been recently adapted for veterinary use. Special attention is focused on dogs which are exceptionally responsive to the social cues of humans making them unique test objects among animal species. In addition to this special co-evolutionary character, dogs share both their living environment and way of life with humans, which make them the closest animal pain model to humans. Pet animals as scientific objects will *per se* attract ethical inquiries. It is unacceptable to cause harm to or risk the welfare of privately owned animals. However, potential analgesics intended for human use could be tested on pets on the same terms as novel veterinary medicines. The predictability of translational pain research could improve by using privately owned animals as pain models especially when testing novel analgesics.

Impact of FELASA working groups on animal welfare

Lars Wass lecture (keynote)
Ann-Christine Eklöf
Karolinska University Hospital, Sweden

FELASA is a Federation of European Laboratory Animal Associations. This organization has been working to improve animal welfare since it was established in 1978. FELASA places great emphasis on the 3Rs of Laboratory Animal Science 'Replacement, Reduction and Refinement'. FELASA also advocates responsible scientific conduct with animals in the life sciences with particular emphasis on ensuring animal welfare. The establishment of FELASA working groups is one of the most important tasks for FELASA. These working groups provide the possibility to harmonize among the member countries. Many of the working groups have been crucial for promoting animal welfare and for securing high standards to perform good and reliable science. FELASA has produced guidelines and recommendations for more than 15 years. The Working Groups consist of specialists in each of the addressed topics, and are nominated by the FELASA constituent associations, and after going through CVs and recommendations are elected by the FELASA Board of Management. A good example of what a WG has produced is the guideline for health monitoring of rodents, which is very important for the exchange of animals between different institutions and countries. In many research institutes and animal breeders it is compulsory to follow this guideline when importing and exporting animals. FELASA and AALAS (American Association for Laboratory Animal Science) established a liaison a couple of years ago. Three working groups were established and two of them have already pub-

lished recommendations and the third dealing with Harm Benefit analyses will soon be ready. There are now new working groups in the pipeline to start working within this liaison. These guidelines and recommendations have influenced the development of various regulatory requirements in Europe, including those related to education and training, routine laboratory animal activities and animal health monitoring.

Session: We Do for Science

Handling of live animals by air

Heikki Nikamaa
Finnair Cargo, Finland

Finnair Cargo provides safe and quick animal transport services to most direct Finnair destinations (from outside of Finland to Helsinki only). Further information is available from our local Sales/Reservations. Our professional staff are there to help during every step of the way. Animal shipments are generally not available within Finland as no special cargo is allowed on domestic flights.

Jetpak (http://www.finnaircargo.fi/en/cargo/domestic-cargo-in-finland.html) can provide information on goods that can be accepted for transport within Finland. When shipping live animals, it is important to book the flights well in advance, and to ensure that you have all the necessary documents in compliance with the regulations of the International Air Transport Association (IATA).

Purchase a suitable animal container: Purchase a container that is suitable for transporting the animal in question. The container must comply with the minimum requirements of IATA Live Animal Regulations. Finnair Cargo does not accept containers with grating on top/latticed top deck. Please note that Finnair does not provide containers for rent.

Check what kind of food is allowed during transport: Make sure that the food provided for the animal during transport complies with the regulations of the transit and destination countries.

Provide feeding instructions: Provide written feeding instructions when handing the animal over for transport. Make sure the instructions accompany the shipment.

Check the physical state of the animal and notify the carrier: When making the booking, check the physical state of the animal, and do this again when handing the animal over to the airline. Notify the airline if the animal is pregnant or has given birth within the last 48 hours.

Notify the carrier of any medication given to the animal: Notify the airline if you have given any sedative to the animal.

New advances in shipping mouse strains as embryos or sperm

Raija Soininen
University of Oulu, Finland

The mouse is the central model organism for analysis of mammalian gene functions and genetic diseases. Sophisticated genetic tools have been developed to generate mice with specific mutations, and a large number of genetically modified (GM) mouse strains have been generated both in individual laboratories and by large international consortia (see www.mousephenotype.org). It is not desirable or even possible to keep all the mouse strains live in animal houses, therefore cryopreservation and *in vitro* fertilization methods have been developed to preserve scientifically valuable mutant mouse strains in liquid nitrogen in the form of frozen germ cells and embryos. To make these resources available to the entire biomedical research community, central mouse repositories, operating with standardized procedures and highest-quality standards, have been established.

The European Mouse Mutant Archive (EMMA), one of the major mouse repositories worldwide, functions as the repository of Infrafrontier, the European research infrastructure for mouse models (www.infrafrontier.eu). EMMA is one of the collaborators of the International Mouse Strain Resource (IMSR), which provides a searchable database (www.findmice.org) of mouse strains and stocks available globally. The transgenic core facility of Biocenter Oulu, University of Oulu, serves researchers as the Finnish EMMA node.

Disseminating mouse stocks as frozen material offers many advantages over live animal shipments, especially with respect to animal welfare and health status issues. Use of liquid nitrogen (LN2) dry shippers has made the dissemination of frozen embryos and germplasm a simple and reliable procedure. Also unfrozen embryos, thawed from frozen stocks, can be sent to customers, and protocols for shipping germplasm in the absence of LN2 are available. The most recent advance is the transport of unfrozen epididymides from which sperm can be extracted for archiving, decreasing costs and inconvenience compared to live animal transportation. It also offers the potential to rescue unique stocks as will be described.

The welfare of the transport

Robert Leblanc
JANVIER LABS, France

Introduction: The purpose of this study was the analysis of the effect of transport on rodents.

Methods: The mouse strain C57BL/6JRj has been used in this study. This strain is very nervous and is the most used inbred mouse strain for animal experimentation. The study concerns two types of transport: short travel (300 km) vs long travel (1,000 km), with control groups. To evaluate the stress level during transportation, the weight of the rodents, the quantity of food and water consumed and the external appearance of the animals were used as parameters. The transport conditions of the rodents for this study were the same as those used for all rodents delivered by our transport department at Janvier Labs.

Results: This study showed that the transport effect depends on transport duration. The shorter the travel time, the more the rodents are stressed and the more the weight loss is significant, the amount of water and food decreases and, for some animals, the general condition is worse. The longer the travel time, the less the rodents are stressed, the more the weight gain is significant, the amount of water and food consumed increase and the general condition of the animals is good.

Conclusion: Contrary to current belief, the transport of rodents over large distances benefits the animals compared with transport with a short duration. The reason for stress is not the transport, but the change of environment for the rodents.

The effects of transportation on physiology and behaviour of rats

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Utrecht University[1], the Netherlands, Harlan Laboratories B.V.[2], the Netherlands, Free University of Amsterdam[3], the Netherlands

Transportation of laboratory rodents unavoidably causes stress. Most laboratory animals used in research are vendor-bred and transported to research facilities, meanwhile experiencing numerous unfamiliar environmental and psychological influences during transportation such as noises, temperature-fluctuations, handling, shaking, vibrations and smells.

To obtain reliable scientific data from experiments using small laboratory animals, their physiological status is required to normalize/stabilize to a condition which can be defined as baseline. Using stressed animals is likely to result in considerable and unintended effects on research results. We investigated physiological and behavioural parameters before and after transportation, as well as in transported and non-transported animals. In-house transportation and light-regime reversals were also studied. Parameters observed were: bodyweight, food- and water consumption, plasma corticosterone, glucose, creatine kinase, core body temperature, heart rate, blood pressure, locomotor activity and home cage behaviour.

Significantly decreased body weight, water and food intake were observed on the day of transportation in transported animals. The temperature inside transportation boxes strongly correlated with body temperature. Plasma corticosterone levels increased at least up to 16 days after transportation. Female control rats showed decreased glucose levels compared to transported females on the day of transportation. Blood pressure, heart rate and activity showed gender specific de- or increase after transportation. Grooming increased and social interactions decreased after transportation.

With these studies we have demonstrated that there are long lasting, gender specific effects of transportation on physiological and behavioural parameters and that there are additional effects of light regime reversal after transportation.

The Microbiological Considerations When Transporting Live Animals

Alistair Thompson
Surrey Diagnostics, United Kingdom

This presentation will discuss the ways in which various infectious agents could be transferred between the outside environment and animals that are in transport, and also how agents can be transferred between the animals within transport boxes. It will then discuss the different testing methods that can be used to detect if any contamination has occurred and therefore if it is safe to release the animals into your main colony.

VIRASURE Air Decontamination System – Theory, operating principle, safety and redundancy measures and applications in BSL-3/4 environment

Juha Mattila
STERIS Finn-Aqua, Finland

Steam sterilization of bio-hazardous material in autoclaves, and storage and decontamination of effluent, requires uncompromised safety and process integrity. The risks arise from the potential contamination of the surrounding environment during the sterilization or effluent decontamination processes. Applications associated with these risks are categorized in one of four bio-safety levels, with three and four being the most critical in terms of process integrity.

To mitigate this risk conventional methods have utilized the following:

- Multiple electrically heated elements or directly electrically heated tube "incinerator" type structure with flow baffles
- Single or dual vent filter arrangements classified as HEPA (high-efficiency particulate arrestance) or membrane filters that commonly have pore size of 0.22 µm to prevent micro-organisms from spreading out through vent connection during process air exchange.

The VIRASURE air decontamination system is different due to its combined forced hot surface contact, coupled with a heated 0.1 µm straining element, all contained in a controlled fail-safe process environment. This improves the safety and redundancy of the sterilizer decontamination cycle or tank containment integrity.

This presentation will discuss the theory and laboratory testing as the basis of this new process approach as well as the benefits of additional safety and redundancy for sterilizers and effluent decontamination systems in the critical bio-containment environment.

Cephalopods as laboratory animals: Back to the future

G Fiorito[a,c], D Osorio[b] ,G Ponte[c,d]
Biology and Evolution of Marine Organisms - Stazione Zoologica Anton Dohrn[a], Italy, University of Sussex[b], United Kingdom, Association for Cephalopod Research – CephRes[c], a non-profit organization, Italy,
Presentation on behalf of 43 Members of the Management Committee of CephsInAction[d]

On behalf of *CephsInAction* - COST Action FA1301: A Network for Improvement of Cephalopod Welfare and Husbandry in Research, Aquaculture and Fisheries

Cephalopod molluscs are listed in Article 1 of the Directive 2010/63/EU making them the first and sole invertebrates considered in the European legislation regulating the use of animals for scientific purposes. Research on cephalopods can be dated back to the XIX century. Scientific interest in these animals emerged due to the extraordinary richness of their behavioural repertoire and the advanced forms of behavioural and neural plasticity they reveal. The relationship between humans and cephalopods is long-standing creating for them a 'social' dimension including being food 'items' or characters in arts, literature and advertising. The diversity of living forms, physiological adaptations, extraordinary genome complexity – as well as other features - make cephalopods a challenge for the scientific community. Among the about 700 living cephalopod species, only about twenty are currently utilized as "Laboratory Animals", and a few others are exploited for their potential in aquaculture.

Compliance with the Directive 2010/63/EU and the increased concern for animal welfare issues face scientists and regulators with an added challenge. The cephalopod 'community' is relatively small, represented by a diverse set of expertise working on a variety of "Laboratory" cephs-species. This provides an unprecedented advantage that prompts interesting questions for science, education, bioethics and the social dimension.

Cephs*In*Action has an ambitious aim. As COST Action, it fosters an interdisciplinary network of experts to promote sharing of toolsand training, and to increase scientific knowledge to improve the care of cephalopods in different contexts. Cephs*In*Action operates to foster multi-disciplinary and inter-species scientific exchanges to integrate knowledge of wel-

fare practices, and to promote cephalopod research, conservation and public awareness.

Currently, Cephs*In*Action (www.cephsinaction.org) includes 18 countries, and involves more than 150 researchers.

Session: Science for Welfare

Dancing with microbes

Keynote
Pentti Huovinen
University of Turku, Finland

The health effects of human microbiota may be wider than have been assumed. Bacterial microbiota may have an impact in allergies, autoimmune diseases, obesity, type 1 and 2 diabetes and inflammatory bowel diseases. The microbiota regulates also brain functions in mice and breeding in insects. In high-income countries, overuse of antibiotics and changes in diet may have selected for a microbiota that lacks the resilience and diversity required to establish balanced immune responses. The changes in microbiota can have significant effects in human health.

Microbiota

Axel K. Hansen
University of Copenhagen, Denmark

Increasing knowledge underlines that the microbiota has an essential impact on a broad range of animal models, such as models of inflammatory bowel disease (IBD), diabetes, obesity, dermatitis and psychiatric disease in which disease expression seems to correlate with the composition of the microbiota. New bacteria, which have not been part of the cultivable bacterial profile, but which have an essential impact on animal model parameters, have been discovered. Among these can be mentioned segmented filamentous bacteria (SFB), which are heavily involved in the expression of Th17 cells, *Akkermansia muciniphila*, which through its potential for mucin degradation may decrease the incidence of diabetes in animal models, and *Prevotella* spp., which may act in favour of diabetes development in animal models. Stress, dietary fluctuations and other uncontrolled factors may change the gut microbiota of an animal, and thereby change the animal model. Today, the bacteriological part of microbiological quality assurance programmes does not include any microbiota characterisation. The cost for a full sequencing, which enables the characterization of the entire microbiota, is rapidly declining, and soon the cost of routine laboratory animal bacteriology may equal the cost of a more selective sequencing approach. By microbiota standardization we may be able to produce animals with less variation. Such standardisation may be achieved by inoculation of tailor made microbiotas or feeding certain prebiotic diets. This approach should, however, be based upon a knowledge of which microbiota will be preferable for which model; knowledge which is not fully available today. Another approach will, therefore, be to screen animals when used for microbiota sensitive studies and incorporate this information in data evaluation, thereby turning this uncontrolled variation into controlled variation.

The top five things that will ensure a successful germ-free project

Frank Razzaboni
Park Bioservices, LLC, USA

The current focus on the microbiome and in particular gut flora has caused a dramatic resurgence of interest in germ-free and gnotobiotic work in rodents. There has been a rush to respond and to get projects started rapidly often without a full appreciation of the difficulty of the mission. This presentation will focus on five key steps for planning, establishing and maintaining a germ-free colony.

1. Plan
2. Involve the extended group
3. Write the protocols
4. Identify the key husbandry staff
5. Establish & maintain the colony

Each of these five steps will be illustrated with examples and further details.

Developing for and using human relevant *in vitro* methods in biomedical research and safety testing

Tuula Heinonen
University of Tampere, Finland

At present, animal-based tests are the major test systems for assessment of tolerability and safety of chemical substances for regulatory purposes, and for pivotal efficacy testing during pharmaceutical development; they are also used as disease models in biomedical research. A vast number of examples

show that animal tests are not ideal test systems to predict toxicity nor pathophysiological processes in humans. The poor predictivity is seen as a contributing factor to the low success rate of pharmaceutical development; less than 10 % of pharmaceuticals in development phase enter market. Thus, tests and testing strategies that are better at mimicking human biology are urgently needed.

The best predictability may be achieved with organotypic models that mimic the microenvironment of the human tissue. The optimum model contains the relevant human cells in the microenvironment of the tissue it is mimicking. Before launching, the model should be characterized on structural, genetic and functional levels. Furthermore, the performance of the model has to be shown using chemicals with known effects preferably on humans (pharmaceuticals). Finally the test should be validated.

Although it is generally agreed that animal experiments should be replaced with better tools, *i.e.* human cell based tissue models, there are only a few accepted *in vitro* tests available at present. To facilitate and to streamline development the OECD has published a guidance document, and the European Commission has created a validation and approval process. In the presentation the key factors to be considered when developing a standardised *in vitro* test are highlighted, and the present OECD-approved tests and testing strategies with the ongoing developments are discussed.

Regulatory studies – reduction and refinement

Marja-Leena Toivonen
Orion Pharma, Finland

The incorporation of *in vitro* and in *silico* screening studies at early stages of drug development is already a common strategy adopted in the pharmaceutical industry. Such screens have the potential to save companies time and financial resource as further investment into unfavourable/hazardous chemicals is halted early, which in turn can reduce the number of chemicals undergoing later mandatory *in vivo* testing. Beyond that, however, nonclinical studies in animals remain essential to establish the proof-of-concept, mechanism-of-action and safety of the drug candidate before progression in clinical development. *In vivo* efficacy and safety data are required to demonstrate the potential benefit to the patient in relation to the therapeutic window. Animal studies are also required to establish the PK/PD relationship and ADME properties of the new drug candidate in order to translate from animal species used in pharmacology and safety evaluation to the intended human patient or volunteer population.

There is nowadays a complex network of documents issued by international and national regulatory authorities regarding drugs, i.e. regulations, directives, guidance and guidelines that need to be addressed in drug development, despite the efforts by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Regulatory authorities are increasingly aware that animal research has limitations. It is also acknowledged that standard animal studies required to test low molecular weight drugs may not be suitable for biopharmaceuticals and other advanced therapies. A case-by-case approach has been suggested for such therapies in which fewer animal studies are required compared to the conventional approach, but which should focus on pharmacologically relevant models. From a regulatory point of view, reductions in animal use can be achieved by science-based removal of out-of-date legislation and guidance, by revision of existing guidance and by advancing the regulatory acceptance of new 3R testing approaches. Examples are the removal of 'Acute single-dose toxicity' studies and the 'Abnormal toxicity test for product batches that are no longer required. Similarly, revision of the 'S2 Genotoxicity testing' guideline has offered an opportunity to reduce the use of animals by combining genotoxicity endpoints into repeat-dose toxicity studies. Changes to *in vivo* approaches could help to address also other endpoints for which otherwise additional animal studies would have to be performed. Lastly, a draft guidance has been recently issued by EMA to facilitate the regulatory acceptance process of new 3R testing approaches.

Predicting human safety – Relevance of animal models

Tiina Pirttilä
Orion Corporation, Finland

Safety and tolerability of a new candidate drug need to be assessed by nonclinical safety evaluation both as a regulatory requirement and to manage the risk to human volunteers or patients. The toxic effects are characterized with respect to target organs, dose, exposure and reversibility. Nonclinical safety

studies are nowadays planned and designed to represent an approach that is scientifically and ethically appropriate: careful planning and selection of the most appropriate animal species are required. Literature on the relevance of nonclinical safety studies for predicting human safety is sparse: there is little published on proposed molecules which have failed to reach the clinical phase because of significant toxicity identified in the nonclinical phase. A survey, performed by 12 pharma companies back in the 1990s (Olson *et al.* 2000) covering 150 compounds with significant human toxicities identified during clinical development showed 71% predictivity for rodent and non-rodent species combined. The best concordance was for hematological, gastrointestinal and cardiovascular toxicities and the least was for cutaneous toxicity. Since that survey techniques and regulatory requirements have developed leading to incorporation of additional end points in safety studies. A more recent analysis of a profile of toxic effects observed in toxicity studies (Horner *et al.* 2013 and 2014) showed identification of a large proportion of the target organs in studies of ≤ 1 month but also in longer studies (≥ 3 months) a significant number of additional target organs were observed. The most common target organ in both rodents and non-rodents was liver, a target of high relevance for human risk assessment. There are unique target organs picked up in both rodents and non-rodents showing a benefit of using two species for assessing safety. Several ongoing initiatives for data sharing will hopefully give us more tools to assess better the relevance of the current animal models.

A comparison of conventional vs. microsampling methodologies for in vivo pharmacokinetic studies in mice

James Rudge
Neoteryx LLC, USA

In recent years, there has been a drive to significantly reduce the number of animals used for bioanalytical studies. Microsampling techniques reduce the amount of blood drawn per time point and allow serial sampling from the same animal, resulting in an overall reduction in the number of animals used per study. A recent toxicokinetic study by Denniff *et al.* showed almost identical curves when comparing samples taken from a normal blood water work-up (100uL) to microsampling (10 µL) using Mitra™ microsamplers. They concluded that, dry matrix sampling passed international agreed criteria for such a study.

We have conducted a mouse study showing the pharmacokinetic profiles for acetaminophen following intravenous dosing and comparing standard plasma sampling vs whole blood (dried) microsampling using Mitra™ microsamplers. The number of mice used for standard sampling was 21 (3 replicas of 7 time points). The number of mice used for the microsampling study was 3 (7 time points per animal). The results (AUC (-t), CLs, t½, Vd) showed that similar PK curves were generated independent of the sampling technique. The study showed that in a discovery setting, dried matrix microsampling can act as a good alternative to standard plasma sampling and in doing so significantly reduces the number of animals required for DMPK studies.

Session: Welfare for Science

Sounds in the animal room – good or bad?

Hanna-Marja Voipio
University of Oulu, Finland

Acoustic environment and sounds have several effects on animal physiology and behaviour and consequently effect animal welfare and experimental results. Measures from animal rooms show that during the working hours sound levels may exceed 100 dB(A) while during the night and weekends especially the rodent rooms are quiet with only a low background noise level generated mainly by ventilation (35-40 dB). Most of the animal room noise is caused by care activities like cage changing and cleaning procedures, but some animals generate high sound levels also by themselves. Our group has shown that the working method and the material of cages can substantially increase the care noise levels; by modifying the way of working the noise can be decreased significantly, even by 15 dB(R).

Animals hear differently to humans and most of laboratory species hear ultrasounds over 20 kHz. This must be considered when monitoring the acoustic environment and estimating the effects. Furthermore, the rat responds differently to different types of sounds even at the same loudness level, which means that the characteristics of the sounds are important.

Sudden sound exposures disturb animals and there have been attempts to mask these by playing a radio in animal rooms. It has been shown, however, that rats prefer silence although some sounds are

avoided more than the others. On the other hand, some studies are encouraging the playing of certain classical music to cover background noise in the animal rooms.

In conclusion, to improve the animals' acoustic environment, control over the sound levels and types is important. In modern animal facilities with standardized ventilation, lighting and equipment, attention must be paid to the daily care working processes that cause the noise peaks.

Animal husbandry and experimental design

Timo Nevalainen
University of Eastern Finland, Finland

There is a clear tendency for authors to describe methodological procedures down to the smallest detail, but at the same time to provide minimal information on the animals and their husbandry. For study design purposes all major variables affecting the animals must be controlled as far as possible. Factors causing bias or variation are also associated with husbandry. It is crucial to understand which of the factors can be managed with proper controls and which need to be addressed by other means. Our lives and the lives of animals are governed by cycles: the seasons, the reproductive cycle, the weekend-working days, the cage change/room sanitation cycle, and the diurnal rhythm. Some of these may be attributable to routine husbandry, and the rest are biological cycles, which may be affected by husbandry procedures. Other issues to be considered are the consequences of in-house transport, restrictions caused by caging, randomization of cage location, the physical environment inside the cage, the acoustic environment audible to animals, olfactory environment, materials in the cage, cage complexity, feeding regimen, kinship, and the presence of humans. Laboratory animal husbandry issues are an integral but underappreciated part of the experimental design, which if ignored can cause major interference with the results. All researchers should familiarize themselves with the current routine animal care in the facility serving them, including the capability to monitor the biological and physicochemical environment.

Towards mutual recognition of training courses

David Smith
Education & Training Platform for LAS, United Kingdom

In 2014, the Commission published an education and training framework that was endorsed by National Contact Point representatives from Competent Authorities of all Member States. It is published at: http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm

As part of this framework, a European Platform and information portal was proposed and subsequently launched. One of its aims is to help facilitate mutual recognition of Laboratory Animal Science (LAS) education and training within the Member States. The Platform, now designated the 'Education & Training Platform for LAS' (ETPLAS) has been in operation for more than one year and its progress will be summarised.

Lack of mutual recognition of training courses within the EU could lead to animals being used unnecessarily for training purposes if training has to be repeated. Free movement of personnel could also be hindered. Since arrangements for LAS education and training is at the discretion of the individual Member State, there is the risk that different requirements for assuring the quality of E & T courses may arise, particularly since those proposed in the EU working document could be seen as aspirational. This could lead to a variable quality of animal welfare and the science using live animals.

There is agreement by Member States that there needs to be a common approach to assure confidence in the quality of training and assessments being provided.

The Platform has developed a draft set of common principles and information requirements for mutual recognition of training courses in the EU. These have been presented for discussion (and eventual agreement) at the meeting of National Competent Authorities (NCP) in March. A wide review of these proposals by all stakeholders is welcomed. A summary will be presented.

LAS education in the light of the new directive – adaptation and optimization of teaching and training activities

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The implementation of Directive 2010/63/EU (Articles 23 and 24) puts new demands on the teaching and training of persons supposed to carry out procedures on animals (function a), designing procedures and projects (function b), taking care of animals (function c), and killing animals (function d). As these functions replace the former FELASA categories A-D, teaching and training activities need to be revised accordingly in order to ensure that the new demands are met. In addition, there is a need for a system allowing mutual recognition of teaching and training activities in the different EU member countries. The Directive puts emphasis on species-specific knowledge for designers (function c), and that anyone performing functions a, b or d must be supervised until competence has been obtained and that they are continuously trained to maintain and improve this competence. Besides that, the Directive does not provide any specific guidelines for how the teaching and training activities should be organized. The European Commission established an Expert Working Group to develop a common education and training framework for the EU to fulfil the new requirements. This resulted in a working document describing different modules for the specific functions with detailed learning outcomes for each of the modules. These modules and learning outcomes form the basis for the new teaching and training activities. This presentation will discuss some of the challenges associated with the transition from the old FELASA categories to the new EU functions, and introduce the subsequent presentations in the session on teaching and training, in which the implementation of the new requirements will be presented and discussed.

The Nordic Consortium in LAS

Matti Nikkola
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The Consortium for Laboratory Animal Science (NCLASET) was founded in 2012 to help universities and pharmaceutical companies meet the educational requirements initially formulated in the European Directive 2010/63/EU.

The consortium initiative aims to provide Laboratory Animal Science education that

- fulfils the criteria defined in the national guidelines
- is organized on an on-demand basis, with continuous entry to courses
- covers all levels of expertise, from the basic to the advanced
- includes all species used in research for which training is required
- is individualized according to the needs of the course participants
- is harmonized to ensure an even, ambitiously defined quality level
- is based on existing structures and educational resources
- is cost-effective so that resources can be focused on continuous development of course materials and courses
- fully supports Mutual Recognition of laboratory animal training activities

The consortium has jointly developed a number of different theoretical course modules such as Rodents and Lagomorphs, Fish and Amphibians as well as guidelines for practical training and for supervisor training. Additional course modules for other species are being developed during 2015. Currently, more than 10 parties (universities and pharmaceutical industry) participate in this education co-operative. In 2015, the consortium will open a website (NCLASET.org) serving as an information point for the collaboration.

Adaptation of LAS courses to the new functions

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The guidelines for education within animal experimentation issued by the Federation of European Laboratory Animal Associations (FELASA) have been one of the most successful initiatives from this organization. These guidelines categorize animal experimentation staff into the following categories: animal caretakers (A), animal experimenters B), license holders (C), and experts (D). Although only 16 category C courses are formally accredited by the organization a range of courses all over Europe adhere to this system for education of animal experimentation license holders. Since 2013 a revised directive from

the European Union on the use of animals for scientific purposes has been in force, and as part of this the EU has issued a more detailed guidance paper for education within animal experimentation. Animal experimentation staff are now categorized according to their functions, *i.e.* persons carrying out experiments (A), license holders (B), animal caretakers (C), and those who kill the animals (D). The guidance paper also gives directions for the designated veterinarians. The previous FELASA guidelines recommended that educational progress be monitored for a specified duration, *e.g.* 80 hours for a category C course, while being taught a well-defined curriculum. In contrast, the present EU guidance paper describes a long list of learning requirements that the successful graduate from a course must fulfil in order to be qualified for specific functions. In addition, functions should be regarded as species specific. For the course provider this offers the opportunity to provide different courses to participants with different backgrounds. It, however, also increases the need to ensure that course graduates do fulfil the mandatory learning requirements. At the University of Copenhagen we have changed our previous FELASA category C and D courses into EU function A/B/D and designated vet courses. The experiences from this process will be presented.

Education and training of staff – improving the welfare of NHPs in biomedical research

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Advancement of scientific knowledge through biological and biomedical research is critical for our understanding of human and animal physiology and consequently for medical progress. Where no alternatives exist, this research necessitates the use of non-human primates (NHPs).

As NHPs are highly sentient animals, it is commonly and unquestionably agreed that their use in experimental procedures has to follow strict ethical standards to continuously support animal welfare and ensure top-level research. Profound and species-specific education and training of all personnel involved in research is a pivotal requirement for the protection of animals used for scientific purposes and a fundamental objective of the respective Directive (2010/63/EU).

Due to the fact that NHP specific courses have been rare or missing, the EU-funded research infrastructure project EUPRIM-Net - a network of ten publicly-funded primate centres - developed an extensive training and education programme for animal caretakers and technical assistants as well as for scientists and veterinarians. This modular designed programme ensures that all personnel involved in primate research acquire a sound understanding of primate biology and consequently NHPs receive adequate care under appropriate housing conditions (www.euprim-net.eu/network/courses.htm).

Accordingly, the courses cover a great variety of topics, including general primate biology, behaviour, husbandry, medical aspects, environmental enrichment and ethics as well as interpersonal skills to communicate state-of-the-art primate-based research. Moreover, a EUPRIM-Net seminar group created a lecture series on Animal Behavioural Management with a focus on Positive Reinforcement Training (PRT).

In order to contribute to the provision of NHP specific courses required according to the revised Directive 2010/63/EU, EUPRIM-Net has compiled a course following the FELASA guidelines. This special EUPRIM-Net course for category A and B staff is envisaged to receive FELASA accreditation by the end of 2015.

Case-based e-learning model "CASUS" "Health management in pig farms" as a complement tool in the practical veterinary education and advanced training as well as in terms of the 3R's

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University of Veterinary Medicine Hannover[a], Germany; Herdecke University[b], Germany

Using an interactive, case- and web-based tutorial "CASUS - Learning with virtual patients" veterinary students are able to test their individual learning success and prepare themselves for practical procedures and exams. Currently, at the University of Veterinary Medicine Hannover, Foundation Germany various "virtual patients" created with CASUS are offered for preclinical and clinical veterinary education. This project is supported by the Competence Centre for

E-Learning, Didactics and Educational Research in Veterinary Medicine (KELDAT).

The 3R's principles can be supported by using virtual patients. Thus, subsequent practical procedures can be performed faster, safer and with more experience, leading to less stress in animals (refinement). Moreover, fewer animals are needed for the practical exercises because of better theoretical knowledge and suitable preparation of the students (reduction).

The project is focused on pig herd visits and subsequent diagnostic procedures. On farm data from herds with typical pig diseases were collected and transferred to e-learning-based cases (virtual herd visits).

By watching these virtual herd visits students can learn about the principles of herd health management and test their knowledge with regard to clinical symptoms of typical diseases, diagnostic procedures, interpretation of diagnostic results and recommending necessary treatments and prevention programs to the farmer. Six CASUS cases were created on the subject areas of respiratory, intestinal and skin diseases, diseases of the nervous system as well as locomotive disorders and reproductive failure. More virtual cases are in preparation.

Since 2013 a yearly doubling number of students has taken this option of learning and found thereby an improved preparation for tests and practical exercises. On average each student has successfully processed two cases and has invested 40 minutes per case. By evaluation of their own learning success, students were given more confidence and were provided with a complementary way of individual learning.

Session: We do

Animal-welfare bodies

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An animal-welfare body is mandated by the EU Directive on "Protection of animals used for scientific purposes" (2010/63/EU). This sets out a requirement for each breeder, supplier or user to have an animal-welfare body to oversee the operation of the establishment, to follow the development and outcome of projects and to advise staff on animal welfare, 3Rs and rehoming (Articles 26 and 27). To find out how animal-welfare bodies are developing across Europe I carried out a survey of my contacts. The results of this small survey (n=19 establishments across 11 countries) show that differing operating models have been set up to satisfy the requirements of the Directive, both as a result of requirements from National Authorities and interpretation at a local level. The various structures in operation will be presented. The results of the survey also show that a wide range of methods have been used to carry out the tasks required by the animal-welfare body. The presentation will give an overview of these with the aim of spreading ideas about common and novel ways in which the tasks can be fulfilled and benefits to animal welfare realised.

The Animal-Welfare Body function: a bureaucratic headache?

Rafael Frias
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The old Directive 86/609/EEC on the protection of animals used for scientific purposes was replaced and significantly updated by Directive 2010/63/EU. The main aim of the new Directive is to firmly adhere to the principle of the 3Rs, and to give top priority to the welfare of the animals that are still needed for scientific and educational purposes. To the specific end of improving welfare, the creation of animal-welfare bodies with the primary task of giving advice on welfare issues was implemented in the Directive of 2010, and thereafter in the legislation that protects laboratory animals in all the Member States since 2013. Moreover, in October 2014 a working document on Animal Welfare Bodies and National Committees was developed by the Commission Expert Working Group. Despite the increased legal and bureaucratic burden for scientists doing animal research, in our experience the introduction of animal-welfare bodies into the legislation has resulted in three main outcomes: 1) an increased communication among all the persons involved with the laboratory animals, that is responsible persons for animal care, welfare and research; 2) a better climate of care in the establishment; and 3) an overall increased animal welfare in the colonies that is directly related to improved quality of science generated from laboratory animals.

Animal Research: Time to talk

Kirk Leech
European Animal Research Association, Great Britain

Animal research remains a contentious issue with a strong vocal opposition. As a result, public engagement by many researchers and institutions remains hesitant and often defensive. This lack of positive communication allows the voices of those opposed to animal research to dominate public discourse. This has the potential to lead to further restrictions on research to the detriment of science, medicine, and society.

For too long, the scientific community (with some exceptions) has allowed the fear of animal rights extremism to prevent its members from speaking publicly about animal research. Today, this fear, although understandable, is increasingly unfounded. While some researchers may encounter animal rights groups involved in vocal but lawful activities, very few will ever come across extremists. These animal rights groups are often large, well-funded organizations with professional advocates, lobbyists, and media consultants, who can successfully command public discussion on the subject of animal research (often with misinformation and unfounded opinion); particularly in the absence of public communication from the scientific community. As a result, members of the public are rarely exposed to a comprehensive, well-informed, and balanced overview of the subject.

Greater openness on the use of animals in research can encourage public trust and allow the scientific community to speak with a united voice. In doing so it can prevent individuals and organizations from being isolated. Pro-active communications will help to garner support and improve understanding; non-communication will only prolong opposition and mistrust. We all need to play a role in illuminating the complex social issues involved with animal research and its benefits to human and animal health.

The scientific community should not allow those who are opposed to animal research to set the public agenda. The aim of the seminar will be to discuss why we should encourage and practice greater openness about animal research.

FELASA in Future

Heinz Brandstetter
Max Planck Institute of Biochemistry, Germany

The Federation of Laboratory Animal Science Associations (FELASA) represents common interests in the furtherance of all aspects of laboratory animal science (LAS) in Europe and beyond. FELASA was established in 1978 by three national organisations: GV-SOLAS, LASA, and Scand-LAS. Over the last decades, FELASA has evolved into a truly pan-European scientific organization with 20 LAS Associations as members representing 26 countries. FELASA forms a huge network for the exchange of information about LAS in Europe, which is still growing. Rus-LASA and PolLASA were the two last associations joining FELASA in 2014 and 2015 respectively.

FELASA puts the 3Rs of Laboratory Animal Science 'Replacement, Reduction and Refinement' centre stage and advocates responsible scientific conduct of animals in the life sciences with particular emphasis on ensuring animal welfare. This is achieved by:

- publishing guidelines, recommendations and policy documents on topics relevant for laboratory animal science,
- representing the views and opinions of the European LAS community as a stakeholder with the European Commission and the Council of Europe in Brussels,
- maintaining relations with national and international bodies concerned with laboratory animal science in Europe,
- improving and promoting education and training in LAS including its accreditation program,
- organising triennial international congresses together with its constituent associations.

The next FELASA Congress will take place 2016 in Brussels and is organised together with our colleagues from BCLAS and NVP and in collaboration with BV: www.felasa2016.eu.

FELASA relies almost entirely on voluntary work by experts appointed by its constituent associations. Additional expertise, views, input and support are always more than welcome. Therefore, do not hesitate to contact us and offer your active participation: www.felasa.eu; email: info@felasa.eu.

Short oral presentations

Session: We and Science

Effects of different housing conditions in rats on body weight, open field behaviour, water avoidance stress and faecal corticosterone levels

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40 male Wistar rats were single housed in conventional (CON; n=20) or individually ventilated cages (IVC; n=20). On arrival, rats were weighed and placed in either IVC or CON cages based upon body weight. They were kept in the animal room for 35 days without handling apart from home cage bedding change every 14 days, and weighing on days 17, 34 and 44. On day 8, home cage bedding was changed and 24 hours later, all faeces were collected for analysis of corticosterone metabolites (CORTm). This was repeated on day 35. On day 36-38, rats were tested in an open field for 12 minutes. Twelve days later, they were exposed to water avoidance for 1 hour, weighed and the bedding changed. Twenty-four hours later faeces were collected to evaluate the stress effect of the water avoidance.

Results: No differences in body weight were observed throughout the 50 day period. In the open field the CON rats showed significantly more horizontal activity (F1, 38) =7.94, p=0.008), and shorter latency to first entry into the centre (F1, 38) =4.26, p=0.045. The CON rats showed significantly higher CORTm than IVC rats on days 7 and day 34 (p=0.03 for both). There was a significant decrease in CORTm between first and second measurements (p=0.000), suggesting that acclimatization to a new environment takes longer than previously assumed. CORTm measures after the water avoidance test did not differ. There was, however, a significant interaction from before the water avoidance stress to afterwards (F (1, 37) =4.90, p=0.033). Planned comparisons revealed that the IVC group showed a significantly higher rise in CORTm levels than the CON group.

Conclusion: Single housed rats in IVC cages show more anxiety related behaviour in the open field and are more reactive to water stress avoidance as reflected in faecal corticosterone metabolites.

Effects of SPF status and reproductive technologies on the body weight, blood pressure and Fkbp5 gene expression in kidneys of hypertensive rats of the ISIAH strain*

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Effects of conditions during rearing and the influence of assisted reproductive technologies on the development of hypertensive phenotype has not previously been elucidated. The aim of the study was to investigate the long-term effects of embryo culture combined with embryo transfer, as well as rearing in specific pathogen free (SPF) conditions, on genetically determined traits of adult ISIAH rats. ISIAH rat preimplantation embryos were frozen-thawed at the two-cell stage and cultured *in vitro* for 48 h in rat one-cell culture medium (R1ECM) up to the morula stage; finally these *in vitro*-derived morulae were transferred to SPF recipient female rats. The pups born after transfer of non-cultured and cultured embryos were reared in an SPF-vivarium and compared with naturally born ISIAH rats reared in either SPF or conventional conditions. The pups were weighted at the age of 13, 25 and 60 days; blood pressure was measured in all groups by the tail-cuff method at the age of 60 days; thereafter the animals were euthanized and Fkbp5 gene expression was measured in kidneys by RT-PCR. There were no differences in body weight between groups at all ages studied. At the age of 60 days all rats possessed hypertension and their mean blood pressure did not differ between groups. However, Fkbp5 gene expression in ISIAH control rats born in the conventional environment (non SPF) was five-fold higher than in both groups born and reared in the SPF-vivarium. Fkbp5 gene expression in kidneys is considered to be a marker of stress. Our data suggest that reproductive technologies, i.e. embryo culture and transfer do not affect the development of hypertension in ISIAH rats. However, rearing in non SPF conditions caused more stress for these rats and resulted in higher expression of Fkbp5 gene expression in their kidneys.

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Breeding laboratory mice – why do some pups die?

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Successful mouse breeding is a crucial part of providing animals for research. Although loss of single pups or entire litters after birth is a relatively common problem, it is poorly understood. We present results from a project aiming to identify risk factors for laboratory mouse pup mortality.

It is sometimes assumed that primiparous females are more prone to lose their litters. However, when analysing breeding data from C57BL/6 and BALB/c mice, we found no effect of parity on litter mortality. When pup mortality is encountered, the pups are often found partly eaten and there is a widespread belief that females kill their pups. Using video recordings, we observed the behaviour of females in detail from birth of the litter until the litter died, and found no evidence that females actively killed their pups. We also observed behaviour from 24h before to 24h after parturition in females that lost their entire litters shortly after birth and females that successfully weaned their litters. Litter loss was associated with females showing less nest building behaviour before parturition, more parturition-related behaviours and more time outside the nest. Lastly, we investigated how different conditions for nest building influenced maternal nest building. Females given a larger amount of nesting material (3 Nestlets) built maternal nests of higher quality in terms of their size, shape, and non-visibility of the female through and above the nest wall, than females given a small amount (0.5 Nestlet).

The project does not support the assumption that female mice actively kill their offspring. Prepartum nest building and parturition-related behaviour are associated with pup survival and pregnant females need sufficient amounts of nesting material to express nest-building behaviour properly. We recommend monitoring of females around time for parturition to detect complicated parturitions.

Access to shelter in metabolic cages results in less body weight loss for mice

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Introduction: An elevated level of albumin in the urine is a result of leakage across the kidney barrier and the use of 24h urine collection to calculate albumin leakage into the urine is an established measurement of kidney injury in rodent models. The time spent in the metabolic cages is stressful for the animals. The animals are single housed on grid flooring and with no bedding available. Weight loss, lowering of the body temperature and ruffled fur coat can be seen in mice after metabolic caging. Prolonged stress is generally accepted to affect factors such as kidney function and pharmacokinetics, so reducing stress in caged animals would likely result in more reliable research data.

Description: In an attempt to reduce the stress a small plastic igloo shelter was placed in the metabolic cages close to the water bottle. Mice can seek shelter in the house, thus reducing the stress and heat loss from single housing on grid floors. The rounded shape of the igloos prevents the mice from climbing the shelter and urine or faeces accumulating on the roof. The mice were treated with streptozotocin in order to develop diabetes.

Results: In the study not using the shelter the mice lost on average 4.3% of their body weight in the week after caging. In the study using shelters the average weight loss was 1.4%.

Conclusions: The use of shelters reduced weight loss in mice during metabolic caging. Effects are likely due to reduced stress and reduced loss of body heat in mice allowed to seek shelter while single housed in metabolic cages. Use of the igloo did not compromise urine collection.

Protocol for histopathological phenotyping of old and genetically modified mice in two background strains (C57BL/6 and sv129)

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Introduction: Genetically engineered mice are often used to investigate gene or protein function. However, the phenotype can vary depending on the back-

ground strain. Furthermore, many phenotyping protocols include histopathological analysis at an early age, before the manifestation of many different spontaneous diseases. Thus there is a risk of misinterpreting gene or protein function with regard to their role in, for example, spontaneous neoplasia. Lrig3 (Leucine-rich repeats and immunoglobulin-like domains 3) is a member of the mammalian Lrig family, and is proposed to be a tumour suppressor.

Objectives: Here we describe a protocol for screening pathological changes in old female mice with different Lrig3 genotypes, bred in either a C57BL/6 or a Sv129-background, together with preliminary results for liver pathology.

Methods: The experiment was approved by the regional animal ethics committee in Umeå. One-year old female mice were euthanized and sampled at their housing facility. Tissue samples were fixed in buffered formaldehyde, then sent to the National Veterinary Institute (SVA) in Uppsala, Sweden, where the samples were inspected, any macroscopical lesions were described, and some tissues were additionally trimmed. After routine processing, sectioning and staining (Mayers' haematoxylin-eosin) a histopathological evaluation was performed. All histological sections were first screened, then detailed scoring of organs was done. All work with the material at SVA was done blinded for the Lrig3 genotype.

Results and conclusion: Staff training ensured good quality sampling with no live animal transportation and reduced costs. A pilot study indicated that the liver and gastrointestinal tract were primary organs of interest with respect to histopathological differences between Lrig3- deficient and wild-type mice. The preliminary liver pathology results (C57BL/6, n=57; Sv129, n=72) showed that before de-coding for Lrig3-zygosity there were both differences and similarities between the two mouse strains that may be associated with either the background strain or the Lrig3-gene version.

Effect of light colour temperature and intensity on anxiety-like behaviour of c57bl/6j mice

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As light is one of the features of the artificial environment where laboratory animals are reared, it is crucial to investigate the effect of some characteristics of light on animal behaviour. In the present study, the effect of light colour temperature and intensity on the anxiety-like behaviour of mice was studied. Twenty-four male C57BL/6J mice were used for the elevated plus maze behavioural test under specific lighting conditions. There were four different experimental groups of six animals each (n=6). These were tested under cool light (4000 °K) of high (60lux) and low intensity (30lux) and warm light (2500 °K) of the same intensities. These intensities were measured in the centre of the maze. According to preliminary data, mice demonstrated a statistically significant higher level of anxiety-like behaviour when high illumination was used compared to low illumination in both cool and warm lighting. However, when testing was done under cool light mice tended to be more anxious compared to the warm light, as evaluated by the number of entries and the time spent in open and closed arms. It is suggested that the intensity of light is an important factor, which should be taken into consideration as it may influence the results of behavioural studies.

Health Monitoring of rodents in microisolation cages. A rationale approach (with an eye to the 3Rs)

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IVCs (individually ventilated cages) are widely used nowadays with the aim of protecting animals and operators. Health monitoring of these units has always been problematic because each cage is, in reality, an independent unit. Traditionally these systems are monitored with sentinels exposed to dirty bedding, and then tested via serology, microbiological culture and parasitology. This approach relies upon variables such as prevalence of disease, dose of agents that are shed by resident animals, frequency and amount of bedding that is transferred in addition to the susceptibility and receptivity of the sentinels. Advances in technologies have allowed other approaches such as the use of immunodeficient sentinels or reliance solely on environmental monitoring. In addition to the improvements in the area of molecular diagnostics, it is now possible to perform non terminal serological tests in rodents by using Dried Blood Spot technology. This approach, in conjunction with other laboratory techniques, makes

possible non terminal sampling screening of animals. We analysed the goal of screening (quarantine or routine monitoring) and the variables involved. We propose that modern technologies can allow optimal health monitoring of rodent colonies with non-terminal samples from dirty bedding sentinels, colony animals and environmental monitoring, in combination. This can be achieved by collecting a single drop of whole blood for serology, faeces and swab for detection of bacteria and parasites and viruses via PCR; resulting in a really easy collection procedure, enhancing the 3Rs and possibly reducing the overall costs of health monitoring programs.

Intraductal human breast cancer model for rapid validation of cancer therapy responses

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Xenograft models of breast cancer are widely used to study the impact of signalling pathway alterations and therapy responses. However, the currently used models poorly mimic the true nature of the ductal origin of breast cancer initiation and progression to metastatic disease. In this project we will establish an intraductal human breast cancer model, which produces breast cancers that morphologically resemble human ductal carcinomas *in situ* and thereby is a far more realistic model than currently used xenograft models. As compared to traditional genetic mouse models, this transplantable model accelerates research as several independent tumours can be generated from cells that are derived from one donor tumour. The transplantable models are widely used as general surrogate models to study functional alterations in cancer cell signalling. Once palpable tumours appear, a researcher can commence treatment of mice with selected chemotherapies.

Recipient female NOD.SCID mice are injected with human MCF10DCIS.com cells through the nipple to the mammary gland. The primary tumours are expected to appear 4-5 weeks after injection. Metastasises are expected to appear at week 8 and create palpable tumours in later weeks. Primary tumour and metastasis imaging is done by IVIS and by ultrasound.

For the pilot test 15 NOD.SCID mice were injected. The first mice were euthanized 4-5 weeks after injection of cells and the remaining mice were euthanized at week 8 or when they grew palpable tumours. Results of this pilot experiment will be reported and we will discuss the potential use of the model for various experimental purposes. The project is funded by Biocenter Finland and is part of the Tissue Engineered Disease Models (TEDM) consortium (http://www.biocenter.fi/technology-platform-services/model-organisms.html#tedm). Once established, the technique will be available as a research service provided by the Turku Center For Disease Modelling (TCDM) (http://www.tcdm.fi).

Implications of post-operative analgesic regimens in the rat Spared Nerve Injury-model of neuropathic pain

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Introduction: The induction of neuropathic pain-like behaviours in rodents often requires surgical intervention. This engages acute nociceptive signalling events that contribute to pain and stress post-operatively that from a welfare perspective demands peri-operative analgesic treatment. However, the majority of researchers avoid providing such care based largely on anecdotal opinions that it might interfere with model pathophysiology in the longer term.

Objectives: To investigate the effects of various peri-operative analgesic regimens, encapsulating different mechanisms and duration of action, on the development of post-operative stress/welfare and pain-like behaviour in the Spared Nerve Injury (SNI)-model of neuropathic pain.

Methods: Starting on the day of surgery, male Sprague-Dawley rats were administered either vehicle (s.c.), carprofen (5.0mg/kg, s.c.), buprenorphine (0.1mg/kg s.c. or 1.0mg/kg p.o. in Nutella®), lidocaine/bupivacaine-mixture (local irrigation in the incision site) or a combination of all analgesics, with coverage from a single administration or up to 72 hours. Postoperatively for 28 days body weight, food consumption and fecal corticosterone were monitored for assessing post-operative stress and recovery, and hind paw mechanical allodynia was investigated for assessing the expected development of neuropathic pain.

Results: None of the analgesic regimes compromised the development of mechanical allodynia. Unexpectedly, the combined treatment with 0.1mg/

kg s.c. buprenorphine and carprofen for 72 hours and local irrigation with lidocaine/bupivacaine, caused severe adverse effects with peritonitis. This was not observed when repeating the combination treatment but incorporating a lower dose of buprenorphine (0.05mg/kg, s.c.), or when buprenorphine was administered alone (0.1mg/kg s.c., or 1.0mg/kg p.o.) for 72 hours. We occasionally observed wound-dehiscence and self-mutilation behaviour, underlining the need for balanced analgesia.

Conclusion: Post-operative analgesia does not appear to affect neuropathic hypersensitivity in the SNI model. However, further studies are needed to determine which treatment is most beneficial to the animals, and to investigate possible effects on the neurobiological mechanisms involved in development of the neuropathic pain.

Making the best of your study with the Göttingen Minipig

AC Søndergaard, A Zeltner
Ellegaard Göttingen Minipigs A/S, Denmark

In the selection of the most appropriate animal species for a study, the Göttingen Minipig should be included as an option on equal terms with other non-rodent species. Ellegaard Göttingen Minipigs A/S is a global developer, breeder and distributor of Göttingen Minipigs for biomedical research. The Minipigs are bred microbiologically defined in barrier facilities fulfilling the industry's wish for an animal model with a high health status. The high health status combined with the strict genetic breeding-management provides our customers with the best opportunity to achieve valid scientific results. With every animal study comes an acclimatization period of appropriate length. Socialization, training and patience are key during this period. Also establishment of good acclimatization procedures are important as confinement stress and poor handling not only decrease animal welfare, but could also be a potential bias for the outcome of the study. This presentation will inform you on the needs of the Minipigs including acclimatization, husbandry, housing and how to make the most of your work with the Ellegaard Göttingen Minipig.

Reuse of slaughter pigs for fresh tissue for *ex vivo* models

Hanne Gamst-Andersen, Kirstine Øvlisen, Peter Lund Gade, Hans Rasmussen, Eva Schandorff Klauman Krøyer, Torben Jørgensen, Gitte Bolt Nielsen, Peter Buhl, Tine Thorup Mellergaard, Susanne Schéele, Emma Goodwin
Novo Nordisk A/S, Denmark

Background: Approximately 400 slaughter pigs are used every year for PD/PK studies of potential new drugs at Novo Nordisk.

Reason for initiative: In several projects a need for fresh tissues exists. For example, fresh pieces of porcine intestine are used for the establishment of *ex vivo* models. Likewise fresh blood vessels are needed for an *ex viv*o method investigating the permeability of peptide drugs across blood vessel walls, and recently euthanized pigs are needed for skin injections investigating insulin pen needles. Another example is excision of skin biopsies from pigs injected under anaesthesia with rapid-acting insulin analogues a few minutes before euthanasia for evaluation of mode of action.

Description of initiative: The opportunity for researchers to get access to fresh tissues regularly has been organised and carried out by employees in the large animal facility by coordinating reuse and thereby eliminating the need of purchasing animals solely for this purpose. Often tissue from one pig could be used for two projects at the same time. Furthermore occasional surplus animals allow access to "naïve" tissues when necessary.

Impact of initiative: In the period November 2013 to November 2014 tissues from at least 70 slaughter pigs, initially used for PD/PK studies, were sampled for *ex vivo* studies.

Purchase of approximately 70 more pigs would have been necessary, if no pigs had been coordinated for reuse.

Future implications: Based on an increasing need of large laboratory animals for PD/PK studies at Novo Nordisk, in the future it will be possible to expand the reuse of large laboratory animals depending on the need for tissues and animals for terminal studies.

In research units with a frequent use of large animals for *in vivo* studies reuse coordination can be applicable and support the development and use of *ex vivo/in vitro* methods by making fresh tissue easily available.

Cardiovascular assessment in NHPs using Jacket telemetry results in high quality data – implementation of modern implants in safety pharmacology studies improves animal welfare

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Novo Nordisk uses non-human primates (NHPs) for research and development investigations where no alternatives exist.

In toxicity studies, measurement of electrocardiograms (ECGs) is a mandatory regulatory guideline requirement and in the past this procedure was carried out in restrained animals resulting in ECG data of low quality. Within the last few years jackets have been developed for measuring ECG in trained NHPs which are less stressed; this improves data quality from animals which can still be pair or group housed. Jacketed ECG procedures do not exclude restraint and stress, however it is considered less than the stress induced under the restrained ECG procedure, and the improvement of data quality is considered to be an overall refinement.

Dedicated cardiovascular safety studies have until now been conducted in freely moving, single housed animals using transmitter implants. Single housing during cardiovascular recording was needed to avoid cross talk between transmitters. Modern implant telemetry allows for group housing due to the use of transmitter implants with different frequencies.

Results: In toxicity studies using jackets and pair or group housed animals, heart rate was significantly lower than in restrained animals. Heart rates above 200 bpm from restrained animals indicate highly stressed animals and cardiovascular assessment of drugs is limited and almost impossible. Freely moving animals trained to wear jackets have heart rates around 125 bpm improving data quality and better cardiovascular assessment.

Video recordings from a study by a collaborator show that animals are highly socialised and non-stressed when wearing jackets measuring cardiovascular parameters.

Conclusion: 1) It is possible to train NHPs to wear jackets for measurement of cardiovascular parameters for better data quality. 2) It is possible to pair or group house during data acquisition. 3) It is possible to analyse cardiovascular data from non-stressed animals and refine assessment of safety in NHP studies.

Moral stress in animal research: a case study of mice breeding

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Recent studies have shown that empathy plays a crucial role in ethical evaluations of animal research and also that many committee members experience moral stress during the project review process. In this presentation we will investigate whether or not knowledge about mouse mortality might cause moral stress among animal technicians involved in breeding and evaluation processes.

Medical research using mice as models depends on a breeding process where large numbers of mice are born. Studies show that large numbers of animals die during the breeding process, in the most commonly used strain (C57) between 13 and 30 % of the mouse pups die during the first days after birth. Although this raises ethical questions, it is seldom discussed as part of the ethical project evaluation required for animal research in the European Union, in spite of the Directive 2010/63/ EU being applicable also to breeding if it causes suffering (Article 1,3).

We are interested in whether this breeding situation is a source of moral stress among animal technicians. As a point of departure we take two situations potentially causing moral stress. The first one is related to the fact that emotional attachment and empathy is facilitated by proximity to the other, including animals, explaining why the technicians seem to be more emotionally affected by the actual research than scientists. The second situation is the process of the project evaluation. Technicians appear to experience a greater amount of emotional turmoil in the process of approving research applications compared to scientists. The discrepancy between knowledge about suffering and not being able to take it into ethical account is potentially stressful. It might also affect the many technicians not involved in the project evaluation but working with the projects, to know that the ethical assessment might have partly overlooked the costs.

Poster presentations

An intravenous catheterization method for mice undergoing dynamic microPET/CT imaging

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Dynamic PET is a very important imaging technique employed for quantification of various physiological parameters (glucose metabolism, cerebral blood flow, etc.). For the injection of the radiopharmaceutical, the mouse must remain still and anesthetized. An additional problem to be addressed for dynamic PET is the position of the animal inside the PET gantry so as to have access to both the eye and the tail. To overcome these difficulties we have modified an existing technique for mouse tail catheterization/injection. In our Institute, we catheterize the lateral coccygeal vein by using a handmade catheter in order to inject the radiopharmaceutical and start dynamic PET acquisition simultaneously. At one end of the catheter tubing, we place the metallic part of 27G needle while the other end is attached to a female Luer lock connector. To remove air, the catheter and the connector are filled with heparinized N/S solution. Topical skin adhesive is applied to secure the extension of the catheter in place. For the micro dynamic PET, the radiopharmaceutical is administered through the i.v. catheter manually, within 10sec. When computed tomography (CT) acquisition with contrast agent is needed after the dynamic PET, the CA is administered through the luer lock connector. Additional advantages of using this catheter are the lower risk of administering an air bubble intravenously caused by switching syringes. Moreover, the total volume of substances is significantly reduced and their loading into the connector can be performed easily without moving the animal.

Team working in laboratory animal science

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Introduction: A well setup organization and well implemented management in laboratory animal science present the benchmark that will enable the creation of numerous teams taking care of constant progress in scientific development. The quality of the results depends on objective definition, team forming, team collaboration and proper exchange of information about specific needs and demands and previous results.

Objectives: are positive, risk and damage controlled outcomes, clarity of goals and achievements, implementation of quality information and results, applicable organizational methods and well based general insight of team members in the process.

Methods: The nature of the research process needs creative management, open paths and adjustable methods, considering the development and different aspects in society. The balance relies on organizational methods already established in practice. Since laboratory animal science presents lively organization, teamwork presents a crucial point in applied management and its methods.

Results: Results of proper management are less time consumption, avoiding mistakes that would potentially lead to economic or other risks. Quality management leads to the continuance of the positive work flow, stronger motivation and satisfaction of personnel involved, resulting in well-based findings and high quality results.

Implementing systematic use of enrichment for chickens

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The Department of Experimental Medicine is implementing the systematic use of enrichment for chickens to improve welfare and to prevent undesirable behaviour in the flock. Maintaining a harmonious population in a small indoor flock of chickens in a research setting poses a great challenge for the caretakers. By taking advantage of the chickens' foraging behaviour and natural curiosity, several novel types of enrichment have been developed and implemented. Using a plan to ensure a systematic change in the types of enrichment during the week helps to achieve better animal welfare.

The effect of environmental enrichment on stress symptoms in male mice during timed mating

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Introduction: It is well known that stress can have an impact on the outcome of research studies in animal models. One way to handle this is to consider the environmental enrichment offered to the laboratory animals.

Objective: The aim of this study was to investigate the correlation between the level of environmental enrichment and the stress level of single housed BALB/cJ mice.

Methods: Male mice were divided into 4 groups. Group A: Enriched with extra nest material hanging from the lid, hazelnut, gnawing stick and single-use house. Cage-change frequency: One week. Group B: Enriched as Group A, but with cage-change frequency of 3 weeks. Group C: Enriched with nest material, gnawing stick, single-use house and a cage-change frequency of one week. Group D: Enriched as Group C, but with cage-change frequency of 3 weeks. The mice were scored once a week for aggressiveness, stress behaviour and nest building on a scale from 1-5.

Results: The mean nest building score of male mice with extra nesting material (group A and B) was significantly better compared to male with less nest material (group C and D), regardless of the cage-change frequency. This indicates that extra nest material may lead to less stress as manifested by better nest building, whereas the cage-change frequency seems not to influence the stress level of the mice. However, no difference in aggressiveness, plug rate or weight gain could be observed between the groups, indicating that extra environmental enrichment does not greatly affect the stress level of the mice. Regardless of environmental enrichment, all mice were scored more aggressive 10-11 weeks after initiation of the experiment.

Conclusion: In this study, extra environmental enrichment only led to better nest building, but had no impact on aggressiveness, plug rate and weight gain, indicating a limited effect of environmental enrichment on stress.

Measurement of iohexol from canine plasma: comparative analysis between enzyme-linked immunosorbent assay, neutron activation analysis and high performance liquid chromatography.

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Iohexol is a non-radioactive, iodinated, water-soluble radiographic contrast medium that is widely used for imaging purposes in both the clinical and research settings. Iohexol is also commonly used as a marker for glomerular filtration rate in both humans and animals such as dogs and cats, and as an intestinal permeability marker in humans, dogs, horses and rats. The aim of this study was to determine the FIT-GFR Iohexol Kit (ELISA) accuracy for the measurement of iohexol in canine plasma, and to compare it to both high-performance liquid chromatography (HPLC) and neutron activation analysis (NAA). Blank and iohexol-containing blood samples (n=100) from dogs were collected from the jugular vein in lithium heparin tubes before and after intravenous application of 3.0 g iohexol/dog to the cephalic vein. The results of this study showed that the correlation coefficients when comparing ELISA vs. HPLC (r=0.98), ELISA vs. NAA (r=0.99) and HPLC vs. NAA (r=0.98) were all highly significant. We conclude that measurement of iohexol from canine plasma using the FIT-GFR Iohexol Kit is as reproducible and precise as using HPLC or NAA. Nonetheless, using FIT-GFR Iohexol Kit for measuring iohexol may be more practical, economical and useful than using HPLC or NAA.

Measurement of iohexol from equine plasma: comparative analysis between enzyme-linked immunosorbent assay and high performance liquid chromatography.

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Iohexol is a non-radioactive, water-soluble, iodinated radiographic contrast medium commonly used in medical imaging. Iohexol is frequently used as a marker for glomerular filtration rate in both

humans and animals, and more recently it has also been used as an intestinal permeability marker in humans, horses, rats and dogs. The main objective of this study was to determine whether the FIT-GFR Iohexol Kit (ELISA) may be used for the measurement of iohexol in equine plasma, and to compare such results with the high-performance liquid chromatography (HPLC), which is regarded as the gold standard for measuring iohexol. Blank and iohexol-containing blood samples (n=100) from healthy horses were collected from the left jugular vein in vacuumed clot tubes before and after nasogastric tube application of 1.0 mL iohexol/kg as a 10 % solution. Results from this study showed that the correlation coefficient when comparing ELISA vs. HPLC was highly significant (r=0.92). We conclude that the measurement of iohexol from equine plasma using the FIT-GFR Iohexol Kit is as reproducible and accurate as using HPLC. Furthermore, it was concluded that using FIT-GFR Iohexol Kit instead of HPLC for measuring iohexol is more advantageous for practical and economic reasons.

The use of a redesigned custom made collar for pigs improves animal welfare and measurement precisions

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Introduction: Our experiments require placement of equipment in an immobilized Tensoplast® collar around the neck of the pigs in order to monitor the site of drug injection. Placing the collar can be stressful for both pigs and technicians, and we have therefore designed a new collar.

Method and Results: The equipment consists of a mounting bracket and a counter. With the old procedure, the mounting bracket is immobilized using Tensoplast® which is formed as a collar around the neck. In order to apply the Tensoplast® collar the pig must be anaesthetized with propofol.

The counter itself must be placed on the mounting bracket after the pig has been dosed with drug. The procedure is not always straightforward, as the pig moves around in the pen. It is very important that the counter is started immediately after dosing. Removing the Tensoplast® collar after the experiment can cause discomfort and pain for the pig, as the material sticks to the pig's bristles.

To simplify the procedure, we redesigned the collars. The new custom made collars are very easy to place around the pigs' necks and do not require the use of propofol in order to immobilize the pigs. Two pockets in the new collar contain the counters. Placing the counters is very easy, as the collar offers greater flexibility and allows some movement of the animals. Hence data acquisition can start immediately after dosing.

Using the redesigned collars, we can avoid the use of anaesthesia; this results in less stress for the animals while securing high quality data. The pigs no longer experience pain and discomfort associated with applying and removing a collar made of Tensoplast®, as the new collar makes this procedure very fast and easy.

Conclusion: We have shown that by optimizing the procedures/equipment, we are able to improve animal welfare to the benefit of both animals and personnel.

A shortened high fat feeding period improves the post-operative recovery in rats with permanent vascular catheters

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In our studies we use a novel technology, Open-Flow Microperfusion (OFM), in which probes are implanted into the subcutaneous adipose tissue (SAT). OFM can be used to continuously assess interstitial concentrations of e.g. insulin in SAT and to investigate the relationship between its pharmacokinetics in plasma and the interstitial fluid.

The original protocol was transferred to Novo Nordisk through an external collaboration. Studies were performed in rats fed a 60% high fat diet (HFD) for 9 weeks to ensure a sufficient amount of SAT for insertion of the OFM probes. Since the OFM technique is combined with an euglycemic hyperinsulinemic clamp the rats also have permanent vascular catheters implanted.

High fat (HF) feeding compromises the normal physiology and welfare of rats, and this negative impact will increase the longer rats are on HFD.

We wanted to investigate whether the period of HF feeding had impact on the post-operative recovery in rats with permanent vascular catheters and whether this period could be shortened, to improve

animal welfare and presumably minimize distress for the rats.

Results: Rats were allocated to two groups fed a HFD for 6 and 9 weeks, respectively. Body weight gain was calculated and the post-operative recovery time was determined by extrapolation. We found that the post-operative recovery periods were 13±1 (6weeks) and 21±4 (9weeks) days, respectively. The amount of SAT was determined by post-mortem examinations.

Conclusion: Rats in the 6 weeks group recovered significantly faster from surgery compared to the 9 weeks group ($p < 0.05$). Post-mortem examination revealed that 6 weeks HFD was sufficient for insertion of the OFM probes and hence the experimental model was not compromised. In summary, reductions of the pre-operative HFD period from 9 to 6 weeks lead to a reduction in the post-operative recovery time.

Practical education of laboratory animal caretakers in Denmark

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The education of laboratory animal caretakers in Denmark is a comprehensive technical education programme spanning almost four years. The education is alternated between periods at a technical school and practical periods as trainees at a workplace with lab animal activity. The Department of Experimental Medicine at the University of Copenhagen is the largest provider of practical education of laboratory animal caretakers in Denmark, and is striving towards offering the best possible practical education of future lab animal caretakers and technicians. The time spent by the trainees at the department is therefore divided between regular work in the animal facilities, participation in customized courses offered by the department and assistance in the teaching and training activities at the faculty. This has turned out to be a successful way of training the caretaker trainees, who are highly motivated to learn and deepen their knowledge in the relevant subjects, and generally complete their education as highly skilled technicians. The aim of this presentation is to describe how the practical education is organized at our department in detail, which hopefully could give inspiration and useful ideas to others responsible for educating animal caretakers and technicians in the Scand-LAS member countries as well as elsewhere.

A global guide to 3R resources

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Norecopa[1], Norway, Animal Welfare Information Center (AWIC), National Agricultural Library[2], USA, Bitfarm[3], Norway

The growth of the internet has resulted in a bewilderingly large number of websites listing databases, information centres, guidelines and regulatory policies which may be of relevance to researchers attempting to implement the 3Rs of Russell & Burch. The majority of these lists merely give links to the resource's website, leaving it up to the reader to assess the relative importance of the individual items.

Norecopa (www.norecopa.no) in collaboration with AWIC has recently constructed a database, called 3R Guide (www.3RGuide.info), which contains quality-controlled information on global databases, information centres, guidelines, journals and email discussion lists. We have also just completed a new, intelligent search engine (search.norecopa.no) which combines a range of search tools to allow the user to identify quickly the most relevant resources. This search engine is at present located on its own domain but will in the course of 2015 become part of a brand-new website for Norecopa. These resources should make it easier for researchers, project leaders and animal welfare bodies to comply with the requirements of Directive 2010/63/EU to use 3R-alternatives wherever possible, and to document their literature search.

Lighting environment: What colour of light do mice prefer?

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Light is an important environmental factor, which influences housing conditions of laboratory mice. Although several characteristics of light such as the intensity and the photoperiod have been extensively studied, very little is known about the preference of mice for different colours of lighting. The aim of the study was to investigate the preference of mice for different light colours. For this purpose, two compact fluorescent lamps of the same characteristics, but of different colour were used; a lamp emitting warm light (2500 °K) and a lamp emitting white light (4000

°K). Sixteen Balb/c male mice, eight weeks of age, were used. Each animal was introduced into a home-made preference setup consisting of two different compartments with one cage in each, connected by a transparent tube. In each compartment a different lamp was used and each animal was free to explore and express its preference. The activity of mice was video recorded for 12 hours. Two observers manually scored the preference of mice. Mice showed a statistically significant preference for the warm light during the first 3- and 6- hour testing period, ($p<0.05$). A tendency towards the warm light during the full 12 hour testing period was also expressed ($p<0.10$). Further preference and behavioural studies are in progress to better define what kind of light colour the mice prefer and to investigate any potential behavioural changes.

Body weight change correlated to causes for euthanasia in old C57BL/6 and Balb/c mice

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Introduction: In mouse studies weight loss is a common end-point. Body weight change is easily monitored and adult mice only lose weight when they have a health problem. However, weight loss can be masked by e.g. tumour growth, and there are diseases that do not cause weight loss or other easily detected symptoms.

Methods: The experiment was approved by the regional animal ethics committee in Uppsala. Mice from strains (C57BL/6: 62 females, 68 males; Balb/c: 80 females, 73 males) bred and housed at the SVA animal facility were used. The mice were weighed once weekly from weaning until euthanasia. Mice were euthanized when 1) weight loss was > 5 % in one week, 2) as matched controls for animals euthanized due to weight loss, 3) moderate symptoms of disease were observed, 4) a successive weight loss of < 5 % per week occurred over four weeks or more or 5) at two years of age. Euthanized mice were autopsied and sampled at SVA. Tissue samples were fixed in buffered formaldehyde, trimmed, routine processed, sectioned, stained (Mayers' haematoxylin-eosin) and subjected to histopathological evaluation.

Results and conclusion: Successive weight loss was more common in C57BL/6 mice (females 16 %; males 24 %) than in Balb/c mice (females 4 %; males 18 %). Many mice were euthanized due to causes other than weight loss (e.g. fighting, piloerection, depression and inactivity, anal prolapse, swollen abdomen) (females: C57BL/6 27 %, Balb/c 35 %; males: C57BL/6 37 %, Balb/c 52 %). This shows that many diseases do not affect body weight in mice, and work is in progress to investigate this further.

Long term substance delivery in chronic dosing studies – Use of polymer based implants for drug release

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Introduction: Chronic dosing of compounds is needed in preclinical efficacy and toxicology studies. Hormones are typical compounds used in chronic dosing. Several dosing methods have been applied to achieve cost-efficient, ethical, stable and long-term release of compounds. Subcutaneous pellets containing solid compound and systems based on osmosis have been widely used. We have developed a polymer-based substance delivery matrix named as MedRod™, which can provide cost effective and stable drug release from weeks to several months in either *in vitro* or *in vivo* preclinical studies.

Objectives: To study the release of dihydrotestosterone (DHT) and 17β-estradiol (E2) from MedRod™ matrix in *vitro* and *in vivo*.

Methods: DHT and E2 were embedded in the polymer matrix and 3mm OD rods were produced. The release of hormones was studied in a dissolution *in vitro* test and animal studies with different mouse strains. ELISA based DHT and E2 immunoassays were performed to quantify hormone levels.

Results: The initial burst release characteristic from polymers was mild and observed within the first days of experimentation in both *in vitro* and *in vivo* studies; after this the overall release profiles of DHT and E2 were stable for several months. In the animal experiments, the DHT levels in mice exceeded significantly the endogenous DHT control levels. Increased DHT exposure induced an increase in hormone-sensitive organs (prostate, liver). Correspondingly, E2 levels were elevated during the study and an increase in the weight of hormone sensitive organs was noted, as expected. Toxicological findings were not observed in the treated animals.

Conclusion: The results demonstrate the benefits of using the MedRod™ polymer-based substance delivery in preclinical animal studies. Benefits include decreased handling stress for the animals and decreased variability in results with lower costs.

The decreased stress and increased accuracy follows closely the 3R principle (Replacement, Reduction and Refinement).

Breeding of mice in the setting of rock blasting

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Animals are sensitive to ground vibration, particularly in the low frequencies 1-100Hz. These vibrations can be generated during rock blasting. The Karolinska Institutet (KI) in Solna, Sweden has been a site for several major construction projects, constructions that have involved heavy rock blasting. During previous constructions (2009-2012) several animal facilities at KI, including Animal facility 1, perceived that the blasting caused problems with breeding of mice, even loss of sensitive strains. With the exception of one animal facility, no precautions were taken in order to reduce the effects of blasting vibrations on the animals. The one facility equipped with devices to dampen the vibrations did not report breeding problems.

For the construction of Biomedicum, which will be one of Europe's largest research facilities (ready 2018), heavy rock blasting was to be expected with 80 000m^3 of rock to be removed. Some of the blasting took place less than 100 meters from Animal facility 1. Based on our previous experience we were concerned that this would affect the breeding of our mice. Since there was good experience in one animal facility from using vibration dampening material, all our caging systems were dampened before the blasting started.

We retrospectively monitored the litter size in three strains. Two of the strains were of C57/Bl6 background and one was an immuno-deficient SCID strain. The monitoring was done over a period of one year, six months before and six months after blasting started.

During the time period studied there were no effects of the blasting on littler size. Signs of stressed animals were observed and mice were more aggressive than usual. Both the facility staff and the researchers reported bites during the first months after blasting was initiated. These stress signs waned with time.

Our results suggest that precautions in the form of vibration dampening is effective to prevent negative changes in litter size in mice and may be considered when blasting close to animal facilities.

Ethical evaluation of mortality in mouse breeding

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When breeding mice for research, loss of single pups or entire litters shortly after birth is a relatively common problem, a mortality rate of 30% for the most commonly used strain (C57BL/6) has been reported. Loss of animals makes planning difficult, and is often compensated for by keeping additional breeding animals. Besides the increased workload and cost, this also counteracts the 3R goal of reducing the number of animals used for research. The underlying cause of mouse pup mortality is not fully known, but dystocia sometimes occurs, causing suffering for the female. Dying from cold or starvation is further likely to cause suffering in the pups. Mice generally give birth during the night, and if parturitions are not monitored, this could result in animals suffering for many hours before being detected.

Although this raises ethical questions concerning both the female and the pups, normal breeding is seldom discussed as part of the ethical project evaluation that is required for animal research in the European Union. Unless the breeding is expected to cause suffering, or the breeding itself is part of the experiment, breeding is not subject to an ethical review process. Both from an animal welfare and an animal ethics point of view this raises several questions: How can a proper cost-benefit analysis be performed if certain welfare aspects are excluded? The high mortality rates allowed for mice indicate that the ethical value of such animals, i.e. the intrinsic value of each individual, is generally regarded as irrelevant. In this presentation we first highlight the potential welfare problems related to mouse breeding, secondly suggest that breeding should be monitored with more concern for welfare, and thirdly suggest that the breeding of research animals needs to be more generally included as part of the ethical evaluation process.

Two refinements improving the welfare of pigs undergoing frequent blood sampling in pharmacokinetic studies

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Introduction: This abstract describes two refinements: First, a harness instead of a regular bandage, which improves the welfare and blood sampling in minipigs with central venous catheters. Second, an extension set attached to ear catheters, which improves welfare during blood sampling in slaughter pigs.

Objectives, methods and results: After surgical insertion of central venous catheters in minipigs, the incision wounds and catheters were protected by a Tensoplast® bandage. This can cause skin irritation and discomfort in the minipigs. One week after the surgery, the wounds were healed and the bandage was replaced with a dog harness placed upside down. The harness was placed loosely on the minipig and, as with the bandage, it was not necessary to immobilise the animal during fitting of the harness. Applying the harness reduced skin irritation and discomfort in the minipig, and thus improved the welfare for the animals. Furthermore, it could be used with social housing, because the harness has a customised pocket protecting the catheters.

The second refinement is an extension set consisting of a 15 cm catheter and a Luer Access Split Septum attached to the jugular catheter. The catheter was inserted via the ear vein in slaughter pigs. The animals were less influenced by withdrawal of blood, because it was not necessary to fixate the ear during blood sampling. Implementing this method resulted in fewer observations of infection around the catheters. In 23 pigs without the extension set, 22 % were treated for infection, whereas none of the 36 pigs with the extension set had infections. The use of this extension set method markedly improved animal welfare by reducing pain and distress.

Conclusion: The two refinements ensure better welfare for pigs in pharmacokinetic studies.

Technical and animal welfare perspectives of portable automated blood sampling with Fluispotter® in Göttingen Minipigs

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Ellegaard Göttingen Minipigs A/S[1], Denmark, CiToxLAB Scantox A/S[2], Denmark, Fluisense ApS[3], Denmark

Introduction: Multiple blood sampling is a time consuming and costly procedure. Often, several sampling occasions in relation to dosing are requested, including sampling outside normal working hours and sampling frequency is often critical. Furthermore, minipigs like other animals are stressed by restraint which may affect certain blood parameters and animal welfare.

Objectives: The aim of this study was to test and evaluate the usability of the newly developed Fluispotter® in Göttingen Minipigs. With the wearable Fluispotter® system it is possible to collect up to 20 blood samples (10µl DBS) in 24 hours without restraining the pig and whilst it is moving freely in its pen.

Methods: Two male Göttingen Minipigs (c. 13 kg) were included in the study. The anaesthetized pig was placed in dorsal recumbency and prepared for catheterization using sterile principles. The sampling catheter was introduced into the right jugular vein through a MILACATH 16Ga (7.5 cm) guide catheter using the Seldinger technique. MILACATH was retracted from the vein, the sampling catheter was secured to the skin and the Fluispotter® was placed in the jacket pocket. Each pig was dosed rectally with 250 mg paracetamol as a test drug and brought to its pen for recovery. On Day 2 the device was detached and the pigs were examined for vascular trauma related to catheterization.

Results and conclusion: During the entire 24-hour study there were only two occasions of restraint (at induction of anaesthesia and at removal of Fluispotter®). Pathological findings (fibrin and thrombin) were related entirely to catherization and catheter displacement inside the vein.

With practice, surgical preparation and catheterization can be carried out by two people in about 20 minutes. Work-load and animal stress during studies with multiple blood sampling is significantly reduced with this new procedure, offering a new tool for Refinement (3R).

Development and validation of an acute delayed type hypersensitivity model in mice using oxazolone

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Introduction: Acute delayed type hypersensitivity (DTH) is a relatively simple and validated method to assess cell-mediated immune function. It is widely used due to its ease of use, sensitivity, and applicability to a wide array of inflammatory pathways. A DTH response on the ear is easy to follow by measuring changes in ear thickness and weight, which are significant and sustained for up to 48 hours.

Objectives: The aim of the study was to develop and validate an oxazolone (4-ethoxymethylene-2-phenyl-5-one)-induced DTH model in female mice. The evaluation included: determining the best responding strain of inbred mice, optimal doses of oxazolone (for sensitization and challenge) and the maximum ear edema reaction after challenge. The model was further validated using the corticosteroid dexamethasone.

Methods: In the DTH sensitization phase, the mice were exposed to oxazolone on the abdomen, followed by a challenge to the same agent six days later applied to the right ear. The left ear was used as control and was treated with acetone. Ear thickness was measured at different time points using an automated micrometer (Digimatic Micrometre, Mitutoyo, Japan). At the end of the study, animals were euthanized and ear biopsies were taken using an 8 mm biopsy punch and ear weight was recorded.

Results: The combination of 2 % oxazolone at sensitization and 0.75 % at challenge gave the most robust ear edema without adverse effects. Balb/c mice were more responsive than CD-1 mice. The peak of ear edema occurred after 24 hours with thickness returning to normal after 72 hours. Dexamethasone showed a strong inhibition of ear edema both in ear weight and thickness.

Conclusion: In summary, we have confirmed that a DTH response on the ear can be induced using oxazolone. The model is robust and could be a good complement for screening anti-inflammatory compounds.