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Abstracts to the 16th FELASA Congress 2025

K21

The Origins of Zoology and Aristotle's Biology

S. Stavrianeas[1]
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Abstract
The study of animal life has been studied since Antiquity with a special interest to the distinguishing characteristic of their purposive behaviour. The capacity of living beings to be active in pursuing certain interests and goals, placed them as the *par excellence* natural beings and the presocratic philosophers of the 6th and 5th century used them as a model for understanding the natural world, the universe, as a whole. But it is with Aristotle that the study of the animal world becomes for the first time a scientific endeavour and a distinct scientific discipline. First, Aristotle's research is exhaustive and relies on a vast collection of empirical data. Second, Aristotle defends in detail a robust scientific method and the explanatory practices that must be employed for the study of life (i.e. living beings and their parts as well as their behaviour). Finally, Aristotle, seeks the origins of the ethical and political dimension of human behaviour in lower living kinds attributing to animals a rudimentary form of ethical virtue, political activity and emotions. In this talk I will delineate the origins of biology and zoology in the Aristotelian corpus, highlighting (a) his field work and classification of data, (b) his method of scientific explanation in the animal world, and (c) his understanding of animals as parts of an integrated natural world. These three aspects of his work places Aristotle as the founder of the scientific study of animal life.

K31

Is the Brain in the Goldilocks Zone?

G. Paxinos[1]
[1]Neuroscience Research Australia and Scientia Professor
The University of New South Wales, Sydney, Australia

Abstract
Atlases are like theories. Like theories, they assist in finding our way in an unknown domain. Paxinos will report on how he used chemoarchitecture as a criterion in identifying nuclei and as a Rosetta Stone for establishing homologies between experimental animals and humans. The maps of the bird brain assisted in producing more accurate maps of the mammalian brain. Comparisons of the brain of humans with that of non-human primates (chimpanzee, rhesus macaque and marmoset) revealed the same nuclei to exist in all primates. Current work involves construction of an MRI/DTI atlas of the living human brain, designed to provide scientists and clinicians similar images to those they view from their subjects/patients. The speaker will reflect on the place of the human brain on the evolutionary tree of brains. Finally, Paxinos will speak of the neuroscience principles behind the formation of heroes of his novel A River Divided, a novel in the environmental genre that links his science to his activism.

K41

An Unexpected Journey – From Calving Cows to Championing Squid Welfare

D. Anderson[1]
[1]LASA, London, United Kingdom

Abstract
Join us on an inspiring odyssey through the remarkable career of David, including the highs and the lows, and offering insights into the evolution of animal care and use in scientific procedures.

From humble beginnings on a bustling farm-based university practice, David honed his skills in teaching and clinical research. This laid the foundation for his pivotal role in navigating the waters of new UK legislation that mandated the appointment of a Named Veterinary Surgeon (NVS) in research institutions. Tasked with initially overseeing an astounding 44 establishments, David embarked on a journey of discovery and innovation in laboratory animal care.

His journey did not stop at the shores of the UK. As an Inspector for the UK regulator, David played an integral role in crafting guidance documents on animal breeding, care, and accommodation. His tireless efforts culminated in his role as General Coordinator for nine working groups that profoundly influenced the revised ETS 123 Appendix A and which subsequently shaped EU Directive 2010/63.

After leaving the UK Inspectorate in 2010, David continued to lend his expertise, guiding the EU Commission in the implementation of key Directive objectives. Though significant strides have been made, he champions a call for more collaborative efforts to tackle the remaining challenges.

David's story is not just of personal achievement but a testament to the power of perseverance and hope. This presentation promises to inspire, offering a unique insight into the convoluted EU legislative framework, and the drive towards improved ethical standards in animal research.

K51

HSBLAS' Growth Chart - the First 30 Years

I. Dontas[1]
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Abstract
The Hellenic Society of Biomedical and Laboratory Animal Science (HSBLAS) was founded in Athens, Greece, in 1995 by a small group of scientists specialized mainly in Veterinary Medicine, Medicine and Biology. HSBLAS' aims were to 1) promote LA welfare and best practice, 2) improve the quality of experimental biomedical research conducted with LA, 3) educate, train and continuously update the scientists and technicians involved in biomedical research including LA breeding, experimental techniques, their optimal pre-, intra- and post-operative treatment, etc., 4) promote the 3Rs, 5) disseminate national, European and international legislation on the use of animals for scientific purposes, and 6) acquire and share knowledge and best practice in the field of LAS within the country and abroad.

While during the first five years the HSBLAS Board was very active in organizing informative lectures and seminars with speakers from Greece and abroad, as well as producing Newsletters with scientific articles, its active membership was less than 30 members. During the years 2000–2010 its Seminars attracted larger audiences, were accredited EACCME-UEMS CME/CPD credits, its Board Members took part in the FELASA Board and FELASA Working Groups, and its membership gradually grew. From 2010 onwards many Veterinarians inspired by LAS joined HSBLAS, and in 2015 a full-fledged LAS Course began, becoming FELASA accredited the following year. HSBLAS has now maturely reached its 30-year anniversary in 2025, which coincides with its achievement in hosting the 16th FELASA Congress in Athens, Greece. Its exponential growth contributes significantly to LA welfare in Greece.

S1H1.1

How Global Animal Activist and Legislative Trends Impact Animal Research

P. Turner[1]
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Abstract
Global animal activist and legislative trends are evolving, for example, impacting dog and primate animal supply to increasing requirements for use of new alternative methods. The in vivo research community around the world is becoming ever more closely intertwined, and legislative or policy changes in one geography can quickly have impacts in other regions or countries. Further, the supply chain is, in some cases, limited to one or two vendors and animal activist targeting of these vendors, their transportation partners and applicable regulations could result in a drastic impact to the field. Another topic concerns legislative changes that may impact the pathway to requirement for alternatives. The European Parliament is under pressure to commit to a roadmap with milestones for a phase out of animal research. In the US, the Food and Drug Administration Modernization Act 3.0 is in a draft stage, which could initiate significant changes to the requirement for use of new alternatives methods. While these trends present challenges, they also promote opportunities for the research community to incorporate more humane considerations for working with animals in science and potentially innovative scientific approaches to addressing research questions and patient needs. The future of animal research will likely see continued advancements in technology and methodology, striving to balance ethical considerations with the need for scientific discovery. This session will explore how the animal activist and legislative trends have co-evolved and what this means for research today and tomorrow around the world.

S1H1.2

Tackling the Legislative Gap for Animals Listed as Invasive Alien Species

N. Dennison[1] and J.M. Fentener van Vlissingen[2]
[1]*University of Dundee, Dundee, United Kingdom*
[2]*Dipl.ECLAM ret, Rotterdam, Netherlands*

Abstract
The EU aims to manage risks imposed by invasive alien species (IAS) that are considered a threat to health, economy, ecosystems and endemic flora and fauna. To that end, Regulation (EU) 1143/2014 was adopted for the prevention and management of the introduction and spread of IAS. Of 88 IAS listed, 24 fall under the scope of Directive 2010/63 EU.

A search by listed vertebrate species in ResearchGate1 identified that most publications on species of concern relate to AIS studied in the wild, both in areas where they are endemic and areas where they were introduced, mostly by human intervention (e.g., pet trade, fur farming, ballast water or sediments). A minority of scientific papers report experimental uses within European research institutions, using either captive bred or wild caught individuals. Recent additions to the IAS list (2022) included two research species, *Xenopus laevis* (African clawed frog) and *Fundulus heteroclitus* (Atlantic killifish). This was recognized by regulators and, to reduce impact on research, an amendment2 was made stating "inclusion of those species …. should be deferred in order to give Member States time to prepare for issuing the permits referred to in Article 8(1)". Unfortunately, there appears to have been little awareness of the upcoming requirements with research institutions or competent authorities in EU Member States.

The overlapping and differing requirements of both bodies of legislation (Directive 2010/63 EU and Regulation (EU) 2016/1141) will be discussed, including containment, the need for qualified personnel and permits, and solutions to potential issues will be suggested.

S1H2.1

Education and Training of the Laboratory Animal Veterinarian Around the World

M. Bailey[1] and G. Borkowski[2]
[1]International Association of Colleges of Laboratory Animal Medicine, Whistler, Canada
[2]AALAC International, Stockholm, Sweden

Abstract
In the rapidly advancing fields of biomedical research and pharmaceuticals, laboratory animal veterinarians play a crucial role in ensuring the ethical and humane treatment of laboratory animals. These specialists are vital to the success of scientific endeavors, bridging the gap between animal welfare and the demands of research. The education and training of laboratory animal veterinarians vary around the world, reflecting diverse approaches to this specialized field, yet all aiming to produce highly skilled professionals dedicated to animal welfare and scientific integrity. Laboratory animal medicine (LAM) is a cornerstone of animal-based research and has been a veterinary specialty for over 60 years. Today 5 LAM Colleges (American, European, Japanese, Korean and Indian) and 1 associate College (Philippines) are members of the International Association of LAM (IACLAM). An IACLAM taskforce investigated the training programs and certification requirements of the different member Colleges. Although there are differences in structure and duration of training and differences in requirements, there are also similarities between the different Colleges. IACLAM's goal is not to standardize training and certification but to achieve global harmonization of education of the Laboratory Animal Veterinarian. In preclinical research involving animals, achieving scientific rigor and high ethical standards is crucial to advancing scientific and medical knowledge, while ensuring animal welfare.

S1H2.2

MSc in Laboratory Animal Science and Welfare - A Comprehensive CPD for Veterinary Specialisation

P. Vergara[1], X. Manteca[1], B. Pintado[2], S. Berdun[3], N. Prats[4], I.A. Gomez de Segura[5], J.A. Fernandez-Blanco[6] and E. Contreras[7]
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[2]Centro Nacional de Biotecnologia/CSIC, Madrid, Spain
[3]Parc Cientific de Barcelona (PCB), Barcelona, Spain
[4]Institut de Recerca Biomedica de Barcelona (IRB), Barcelona, Spain
[5]Universidad Complutense, Madrid, Spain
[6]Parc Recerca Biomedica de Barcelona (PRBB), Barcelona, Spain
[7]Institut de Recerca i Tecnologia Agroalimentaries (IRTA), Barcelona, Spain

Abstract
Laboratory Animal Science (LAS) has emerged as a critical veterinary specialty, playing a pivotal role in advancing animal-based research and ensuring the highest standards of animal welfare.

However, due to the diversity and complexity of the field, extensive and long-term E&T programs are required. In this sense, while the Residency programs recognised by the Colleges on Laboratory Animal Medicine, i.e. ECLAM or ACLAM are very good, they can only cater for a very small number of veterinarians.

For this reason, since 2000, we have developed a modular and flexible MSc in Laboratory Animal Science and Welfare program at the UAB that leads to professionally recognised MSc qualification, and which is open to any working veterinarian wherever they live.

The program includes online sessions together with lectures and practical sessions at the UAB, which are organised for short periods of 1–2 weeks twice a year, thereby enabling students to obtain a qualification while remaining in their jobs.

Thanks to the support of international organisations such as ICLAS, Laboratory Animal Limited and Novartis, since 2000, students from more than 12 countries around the world have enrolled on the course creating a rich, multicultural learning environment that has been enhanced through networking and cross-cultural collaboration.

S1H2.3

Staying True to our Values: Resilience Strategies for Veterinarians Facing Challenges with Compassion Fatigue

N.E. Trimmel[1]
[1]Johannes Kepler University Linz, Linz, Austria

Abstract
Veterinarians working in animal research are guided by deeply rooted values that reflect their unique role at the intersection of science, ethics, and animal welfare. Compassion for the animals under their care drives their commitment to ensure the highest standards of well-being, even in challenging research settings. Their dedication to ethical responsibility aligns with the principles of the 3Rs, requiring them to advocate for practices that minimize animal use and suffering while maintaining scientific rigor. Furthermore, veterinarians value collaboration, acting as liaisons between researchers, institutional leadership, and animal care teams to balance diverse priorities and uphold the integrity of research. This multidimensional responsibility positions veterinarians as key contributors to fostering a Culture of Care within research facilities.

While these values serve as guiding principles and intrinsic motivators, they can also confront vets with many obstacles and increase their vulnerability to compassion fatigue, particularly when vets face moral dilemmas, heavy workloads, or emotionally taxing situations. To sustain their well-being and effectiveness, veterinarians must adopt intentional strategies to build resilience while staying true to their core principles.

This presentation will highlight the key values that often drive veterinarians and explore practical strategies to uphold these values while building resilience in the face of work challenges. The focus will be put on actionable strategies for navigating moral stress, fostering peer support networks, and aligning personal values with institutional priorities. Practical tools, such as reflective practices and self-care strategies, will be discussed alongside the role of leadership in creating a supportive work environment.

S1H3.1

The Making of a Specialist: The Designated Veterinarian eModule 24

N. Dennison[1], A.-D. Degryse[2], M. Lloyd[3] and L. Whitfield[4]
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Abstract
The Designated Veterinarian (DV) is a veterinarian with expertise in laboratory animal medicine and is responsible for providing advice on animal well-being and treatment to those breeding or supplying animals for, or using animals in, research.

The DV eModule is one of thirteen online courses commissioned by the European Commission. It has been designed as an introduction to the role of the Designated Veterinarian and provides insight into the knowledge and skills required to perform effectively in this role. The module aims to deliver a toolbox of knowledge, skills and attitudes to help new DVs navigate the complexities of the tasks required of them and to provide them with "day-one competences" to deal with the wide range of situations that they will need to deal with.

The course covers all thirty learning outcomes outlined in the DV task specific module of the Commission's Education and Training Framework1. It is split into chapters that allow learners flexibility in how they access and complete the module, using scenario-based learning where possible. Topics covered include statutory roles, communication and interactions, specific legislative requirements, ethics, welfare and the 3Rs, in addition to sections based on the more practical aspects of animal health, welfare and husbandry.

eLearning provides flexibility, convenience and cost-effectiveness. The course will be freely accessible to DVs across Europe, and indeed laboratory animal veterinarians elsewhere in the world, supporting new DVs in starting their specialist journey and providing "refresher" training for those currently in post.

S1H3.2

In line, Out of line, Over the line - the many forms of leadership

R. Rose[1]
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Abstract
Laboratory veterinarians often seek advice of council members of professional bodies for daily challenges to career pathways, citing institute organisational hierarchy. From team structure, to role, to job title – many brilliant veterinarians felt they were not achieving. When combined with their day job and societal pressure on the profession, a risk for staff retention is high. Common themes showed a lack of awareness of other potentially more suitable roles/paths available, or commonly a poor understanding of the reality of roles being aspired to. Using the concept of 'vet-led team' and applying this to the laboratory setting helped demonstrate various ways veterinarians could be leaders, and more often than not already were, using three categories. 'In line' formal structures rely upon line management responsibilities with benefits of clear accountability. 'Out of line' may include matrix or contracted roles, relying on influence rather than traditional line management-based leadership. 'Over the line' expands to leading outside beyond day job eg thought/sector leader. Leading is an innate skill all working in laboratory animal care programs have, however data showed many individuals do not take time for self-reflection, seeking feedback or mentorship/coaching. Without this there is little ability to articulate our value if seeking career progression, or to hone skills to increase impact. Demonstrating career pathways or leadership opportunities (including less commonly considered e.g. volunteering for professional bodies) to the organisation members and others in the profession aided progression towards their personal goals whilst staying in the field of laboratory medicine or regulatory welfare compliance.

S1H4.1

Technical Advances and Data Science for Monitoring Animal Health and Welfare

A. Bleich[1]
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Abstract
Animal welfare and refinement strategies in biomedical research depend on the correct assessment of pain, suffering, harm and distress. However, measuring animal welfare and the impact of scientific procedures is not an easy task. It requires objective, evidence-based and applicable parameters, techniques to assess these parameters, ideally integrated into the home cage environment, and data science approaches to translate the measurements into a welfare or severity framework. Much effort has been put into the development of parameters and techniques in recent years, e.g. through the DFG-funded FOR2591 "Severity assessment in animal based research". This talk will review and critically discuss the results of such research, how parameters and models have been systematically evaluated, how data science and AI have been applied, and how existing and new technologies have been integrated to advance the goal of objective assessment and improved animal welfare in science.

S1H5.1

Refinement of Anesthesia, Analgesia, and Euthanasia

C. Pacharinsak[1] and P. Sharp[2]
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[2]Purdue University, West Lafayette, United States

Abstract
A significant portion of animal research involves the administration of anesthesia, analgesia, and euthanasia. The protocols for these procedures can significantly influence or impact the outcomes of a study. Refining these protocols to align with the

specific objectives of a study can be a complex and challenging task. Given the absence of a universally applicable protocol, scientists must consult with veterinarians to ensure that their goals are harmoniously integrated with the chosen protocol(s). Although there is no ideal anesthetic and analgesic protocol for every procedure, anesthetists and veterinarians should possess a comprehensive understanding of the pharmacological effects of drugs and the physiological variations among their animal subjects (including sex, strain, age, etc.). They should also be cognizant of the availability of the necessary equipment and tools for their respective tasks. Regardless of the chosen protocol, employing a balanced anesthesia and preemptive/multimodal analgesia approach is highly recommended. These techniques facilitate the reduction of the dosages of individual anesthetics and analgesics while simultaneously minimizing the incidence of adverse side effects. At the conclusion of the study, euthanasia should be carried out in a manner that minimizes stress for the animals. This presentation will review recent publications pertaining to anesthesia, analgesia, and euthanasia techniques, incorporating our own experiences and expertise in these processes and procedures.

S1H5.2

Evaluating CO_2, CO, and N_2 to Refine Euthanasia Practice and Welfare in Laboratory Rodents

C.F. Moreira[1], P. Villiger[1], C. Calvet[1], M. Halbeisen[1], F. Prisco[1], C.A. Wagner[1] and P. Seebeck[1]
[1]*University of Zürich, Zürich, Switzerland*

Abstract
Euthanasia techniques in laboratory rodents are often assessed through behavioral observations, which may fail to capture critical physiological events like metabolic distress, pain, or the precise moment of loss of consciousness (LOC). Carbon dioxide (CO_2), a widely used euthanasia agent, is associated with adverse effects such as gasping and agitation, prompting the exploration of alternatives, where carbon monoxide (CO) and nitrogen (N_2) are included.

A multifaceted experimental approach combined electrophysiological recordings from the heart (ECG), brain (EEG), and muscle (EMG) using telemetry, blood pressure measurements, whole-body plethysmography, behavioural observations during gas exposure, biochemical analysis of stress markers, and lung histopathology. This extensive dataset allowed us to evaluate CO_2 at 30% and 70% concentrations, CO, and N_2 as option for a more humane euthanasia in mice.

Overall, the results indicate that CO_2 is the optimal killing gas compared to CO and N_2. While the 30% CO_2 setting exhibited some distress markers (e.g., gasping, agitation, delayed LOC, among others), 30% CO_2 was the only experimental setting in which animals did not enter hypoxic conditions prior to LOC, as observed in the 70% CO_2, CO, and N_2 groups. One could further explore the combination of CO_2 with an anesthetic agent during euthanasia to mitigate those distress markers. Ultimately, this study contributes to the understanding of aversive characteristics and potential hypoxic effects from CO_2, CO, and N_2 exposure. In doing so, it offers a more comprehensive framework for current practices aimed at minimizing various forms of distress and refining the welfare of laboratory mice.

S1H6.1

Voluntary Intake of Sedatives before Isoflurane Anaesthesia Induction in Rats - A Sweet Pilot

T. Pettersson Dynesius[1], V. Bettembourg[1] and P. Hedenqvist[1]
[1]*Swedish University of Agricultural Sciences, Uppsala, Sweden*

Abstract
Isoflurane is commonly used for induction of inhalation anaesthesia in rats, without prior sedative premedication. Volatile agents can cause stress when inhaled and are aversive to rats. To minimize stress during induction and recovery from isoflurane anaesthesia, rats can be premedicated with sedative agents. The aim of the study was to evaluate if 25 Wistar rats, 3–6 month old of both sexes would voluntarily consume four different sedatives in Nutella and, how it affected behaviour during induction and recovery from 15 minutes of isoflurane anaesthesia. The rats learned to consume approximately 1 mL of Nutella within four days of training. The sedatives were mixed with Nutella, and Nutella with water was used as control. Filming allowed for blinded evaluation of behaviour by subjective scoring of quality as well as with Behavioral Observation Research Interactive Software (BORIS). Corticosterone plasma levels were measured by ELISA (Tecan) after 10 minutes of anaesthesia. Midazolam (7.5 mg/kg), tasipimidine (0.6 mg/kg), and buprenorphine (0.5 mg/kg) were all consumed within few minutes when mixed in Nutella. Acepromazine (15 mg/kg) markedly reduced time and volume of intake. Only midazolam caused obvious sedation in 2/5 animals. There was a tendency toward better quality of induction with all sedatives compared to controls. Midazolam reduced the corticosterone plasma levels compared to controls. The quality of recovery did not differ between groups. Ethogram-based behaviour analysis was not conclusive. Voluntary oral intake of a sedative agent has the potential to minimize stress caused by induction of anaesthesia with isoflurane in rats.

S1H6.2

Effects of Repeated Anesthesia of Laboratory Mice with Isoflurane, Nitrous Oxide and Sevoflurane

P. Villiger[1], N. Andresen[2,3], M. Halbeisen[1], F. Prisco[4] and P. Seebeck[1]
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[2]*Technische Universität Berlin; Computer Vision & Remote Sensing, Berlin, Germany*
[3]*Freie Universität Berlin; Institute of Animal Welfare, Animal Behavior and Laboratory Animal Science, Berlin, Germany*
[4]*Universtity of Zurich; Vetsuisse Faculty; Institut für Veterinärpathologie, Zürich, Switzerland*

Abstract
Mice are anesthetized for many experimental procedures. Volatile anesthetics are used because of their ease of use, safety margins

and lack of risk of misinjection. Isoflurane is the most commonly used anesthetic gas in research. However, recent studies have raised concerns due to its pungent, irritating odor, which can cause aversion and discomfort in mice, especially when they are repeatedly exposed to isoflurane.

To address this issue, we investigated the repeated exposure to isoflurane, sevoflurane and nitrous oxide anesthesia in C57BL6/J and BALB/c mice (10-week-old, females and males). The anesthesia was induced in a whole-body plethysmography chamber with 4.3% isoflurane in oxygen, 5.6% sevoflurane in oxygen or 80% N2O in oxygen. We collected respiratory, behavioral and ultrasonic vocalization (USV) data during the induction, maintenance and recovery phases. To assess possible differences in (di)stress, we measured plasma corticosterone and used a machine learning based algorithm to score the grimace scale. Except for anesthesia, mice were group housed in a digital ventilated cage (DVC) system to monitor their activity patterns throughout the whole experiment.

Our preliminary data show that the induction of isoflurane and sevoflurane resulted in a reliable and rapid loss of consciousness in all exposures. Mice exposed to 80% nitrous oxide did not lose consciousness at all and we did not observe any sedative sedation effects. We also observed an increase in muscle spasms in the isoflurane and sevoflurane groups. The DVC data showed altered cage activity during the night following the gas exposure (for all three anesthetics).

S1H7.1

Synergizing Veterinary and Scientific Practice in Preclinical Animal Research

C. Brayton[1]
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Abstract
Achieving scientific rigor and high ethical standards is crucial to advancing science, while ensuring animal welfare.

Good veterinary practice focuses on health, welfare, and ethical treatment of research animals. It involves expertise in care and management, and constant attention to humane care and use.

Good scientific practice requires integrity and expertise regarding ethical standards, experimental design, relevant models, experimental conditions, data management and reporting.

Application of the 3Rs requires robust Veterinary and Scientific Practice:

Replace animals with non-animal alternatives wherever possible. e.g. elements of a research question are addressed in silico or in vitro, and **replace** or **reduce** animals by informing study design.

Reduce animals needed for valid, relevant, reproducible outcomes by improving study deign.

Refine procedures to mitigate their severity:

e.g. by **veterinary practices** that **reduce** stressors, morbidity and mortality; by **pathology** that diagnoses disease or adverse outcomes, leading to **clinical interventions** and **experimental refinements** that improve health and wellbeing;

e.g. by **scientific practices** that reduce and refine by selecting relevant models and controls; by applying techniques that achieve study aims, minimize pain and distress, and confounding variables; by applying statistical design to optimize animal use; by applying pathology to validate experimental outcomes; and by reporting models, procedures, outcomes accurately and usefully.

This session explores strategies for synergies in veterinary and scientific practices, to achieve animal welfare and health, and scientific excellence. It aims to foster discussions leading to better practices for care and use, selection, study design and reporting of animals in preclinical research.

S1H7.2

The Path to Better Science: Resources from Norecopa

A. Smith[1]
[1]*Norecopa, Ås, Norway*

Abstract
The daily life of a laboratory animal veterinarian is a constant search for ways to ensure the quality, reproducibility and translatability of preclinical research and testing, maximise the welfare of the animals involved, ensure health & safety, and promote a culture of care between all stakeholders.

Norway's 3R platform Norecopa aims to be a "one-stop-shop" for up-to-date, quality assured global 3R resources for all types of research and testing. Its 10,000 webpages use an intelligent search engine with a large range of filters to facilitate rapid identification of key resources. Norecopa issues 6–7 English-language newsletters each year, to provide a quick overview of the latest global 3R advances.

Efforts to improve the reproducibility of preclinical science have often focused on better reporting. A report can, however, never be better than the quality of the work it describes. For that reason, Norecopa led the production of planning guidelines (PREPARE, https://norecopa.no/PREPARE), consisting of a checklist (in 36 languages) and website of resources. PREPARE was developed over many years, using experience in managing accredited facilities and from discussions on courses in Laboratory Animal Science.

The Norecopa website also contains databases of textbooks and alternatives or supplements to animal use in education and training. We have created a Refinement Wiki for the rapid dissemination of large or small improvements to housing, care and use. Norecopa also hosts the websites of the International Culture of Care Network and the European Network of National Networks of Animal Welfare Bodies (ENAWB).

S2A1.1

Reporting Genetically Altered Zebrafish According to Their Phenotype

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Abstract
Regulatory statements on the creation, breeding, and use of genetically altered (GA) zebrafish are complex. It is essential to define the fish to be considered during the creation of GA models, assess the consequences of these mutations on animal welfare to

determine whether the phenotype is harmful or not. Additionally, the method of sampling for genotyping must be identified, and its regulatory declaration ensured accordingly. Finally, managing harmful phenotypes in breeding programs is a challenge due to the complexity of identifying them in fish.

The new European guide on the use of GA animals provides essential elements for project authorization applications and statistical declarations for GA zebrafish, offering adapted tools.

S2A1.2

Amended Directive 2010/63/EU for Zebrafish Housing and Euthanasia: Swimming to the 3Rs

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Abstract
Directive 2010/63/EU has recently been amended aiming for national implementation by December 2026. The amendment contains zebrafish (Danio rerio) specific requirements and authorisation. Housing standards are detailed and they will have some direct implications on the isolation of adult zebrafish for identification post fin clipping. In practice, the regulation will encourage the genotyping of larvae rather than adults, a significant 3R application that we will discuss. When it is still necessary to fin clip adults, we will describe some protocols to provide analgesia practically. At the end of the animal's life or to sample fish, euthanasia is performed. The amended directive will now authorise to kill zebrafish by hypothermic shock. That does not mean that the method is more refined than an overdose of anaesthesia – it depends on the anaesthetic. We will discuss options for the euthanasia of zebrafish and propose refined options according to the purpose of the euthanasia.

S2A1.3

Genotyping Comparisons for the 3Rs in the Zebrafish Model

C. Allen[1]
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Abstract
Genotyping is an essential tool used to identify customised zebrafish GAA lines. Genetically engineered GAA lines often do not show a discernible phenotype and many are interbreed with multiple alleles, particularly transgenic lines. Both qualitative and quantitative DNA samples are required to track inheritance through genetic identification. Adult fin clipping is a popular method of tissue sampling for DNA extraction, which was not originally considered painful. It is now widely accepted that fin clipping is aversive to the zebrafish, regardless of the size of the clip taken. In a bid to reduce adult fin clipping events and implement the 3Rs, swabbing has been shown to be a refinement to fin clipping. This method impacts fish through netting and handling and unfortunately only gives small quantities of DNA, which can introduce error into the subsequent analysis. Larval fin clipping is a reduction whereby embryos have their developmental caudal fins sampled, which regenerate quickly. Adequate DNA can be harvested from these larval clips for multiple allelic identification. Larval clipping is a crucial tool in the reduction of animal numbers within the zebrafish research world. Research will, however, always require choices. There will be situations where adult fin clipping or swabbing is required because a mixed shoal had to be reared for group dynamics or phenotypic development. Furthermore, errors may occur; in preliminary identification; maintaining separation of fish with certain genotypes; and when returning outcrossed pairs back to their tank. Rechecking adult fish in exceptional circumstances reduces the need for new generations.

S2A1.4

A Guide to Help Furnish and Care for Fish Housed in a Laboratory Environment

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[5]*Swedish National Committee for the Protection of Animals used for Scientific Purposes, Jönköping, Sweden*
[6]*Swedish Board of Agriculture, Jönköping, Sweden*

Abstract
In bodies of water around the world, there are numerous different types of habitats. These habitats are the homes of thousands of fish species, each requiring their own set of living conditions. The natural habitats, from the cold, open sea, to warm coastal areas full of vegetation as well as lakes, rivers and streams, are difficult to replicate in a laboratory setting. EU regulations on animal welfare requires that fish in laboratories shall have access to appropriate furnishings, such as hiding spaces and bottom substrate. Swedish legislation requires the interior to correspond to that found in the natural environment of each fish species, which can cause problems in a laboratory setting. To assist Swedish researchers when deciding on how to create the best interior environment for the fish, the Swedish 3Rs Center has created a guide. The guide contains general considerations and advice on housing fish in a laboratory environment, as well as specific information for 41 species and groups of fish that are being housed for research in Sweden. Included in the specific information is where the species comes from, if they are endangered, how they live in the wild and how they should and could be housed in a laboratory setting. The guide was created with the support from Swedish fish experts with the ambition to assist fish researchers in Sweden and other countries to furnish for animal welfare.

S2A2.1

Homer, Herodotus, Hippocrates and Aristotle: What have the Ancient Greeks Taught us about Animals?

W. Jarrett[1]
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Abstract
As well as being the birthplace of democracy, Ancient Athens and the wider Hellenic world gave us some of the first anatomical studies and ethical thinking about animals. In Homer, animals are sacrificed in their hundreds, but are also loyal companions. Herodotus tells us far-fetched tales of flying snakes and ants the size of foxes. Hippocrates and Galen, the fathers of medicine, both experimented on animals. But perhaps the most systematic studies of animals – their anatomy, how they move and how they experience life – were carried out by Aristotle, pupil of Plato and tutor to Alexander the Great.

Aristotle's hierarchy of living things, with man at the top of the ladder, has persisted into our current thinking around which species are more sentient than others and which should be protected under law.

This talk will examine Ancient Greek stories about, and attitudes to, animals. As well as giving many light-hearted examples, it will explore some of the thinking that is still relevant today, and which has helped to shape modern attitudes to animals and how human beings use them in science.

The target audience for this talk is anyone with an interest in Ancient Greek attitudes to animals, the history of thought about man's relationship with animals, and where our current ethical thinking about animals and animal sentience comes from.

S2A2.2

Why Openness Works

M. Addelman[1], J. Stanley[1] and M. Kamper[1]
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Abstract
Surveys consistently show strong support for the use of animals in medical research. Trust, though, is another matter: a 2018 UK Ipsos Mori poll showed only 30–40% of the public trust organisations as a source of reliable information. The poll followed years of animal rights protests, sometimes violent, often threatening, forcing scientists into the shadows and undermining their efforts to develop new medicines and technologies to tackle disease.

In 2014, that changed with the launch of the world's first transparency agreement, the Concordat on openness on animal research. As one of the original signatories, join us to hear about the huge rewards that openness can bring for science, scientists and ultimately the public who are the beneficiaries of new treatments and procedures, not to mention the animals themselves.

The University of Manchester has become one of the best known Universities in Europe for our communications around using animals in research and we were officially designated as a 'Leader in openness' in 2019. Learn how our communications and engagement has changed minds and fostered debate through our award winning website, social media content, public debates and engagement with young people and other groups. We will also outline how, by working with internal and external stakeholders to develop our culture of openness, openness has positively impacted our culture of care towards our animals and the staff who care for them.

S2A2.3

Talking about Harms, Severity and the Limitations of Animal Research

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[2]RSPCA, Horsham, United Kingdom

Abstract
Recent years have seen increasing awareness and acknowledgement of the need for, and importance of, openness around the use of animals in science. There has also been much reflection around ensuring that information about animal use is both balanced and accessible.

Providing 'balanced' information means being clear about the purposes of animal use; being realistic about the potential benefits; and being honest about limitations of animal research. It also means accurately portraying standards of regulation, science and animal welfare; acknowledging the ethical dilemmas involved; and being open about what animals experience - including the nature and level of any suffering. Talking about harms to animals, and the limitations of animal research, remain particular challenges for many.

There are many opportunities for those involved in funding, breeding or using animals in research to communicate with the public - this includes via an institution's website or social media accounts.

This workshop will discuss what good practice can look like, as well as common shortcomings, and will also highlight helpful initiatives and guidance in this area.

S2A2.4

The Use of Social Media to Demonstrate Awareness in Animal Research

Y. Li[1] and I. Serrenho[1]
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Abstract
Social media is an essential way to inform, educate and unify audiences in support of biomedical research by providing accurate and evidence-based information about the importance of the humane use of animals in biomedical research. The popularity and diversity of social media platforms offers institutions and individuals the opportunity to communicate about animal research with new and hard-to-reach audiences and therefore play a crucial

role in demonstrating ethical awareness and accountability in animal research.

As a pan-European advocacy organisation, EARA has 18 X accounts across Europe. These accounts allow EARA to provide information in native languages, and also help us gain a better understanding of the research that is ongoing in that country. During the EARA-led *Be Open about Animal Research Day* (#BOARD24), on 3 May 2024, over 1000 institutions supported a global social media campaign designed to celebrate openness about animal research, with activity on X, Instagram, YouTube, LinkedIn and Facebook. EARA plans to hold #BOARD25 during the FELASA 2025 Congress, which will bring together participating institutions to communicate openly about animal research, with a focus on Congress streams 1, 3 and 4.

EARA will use this presentation to provide an introduction to attendees on how to take advantage of social media to improve openness and transparency about their work, and how their presence on social media can be used for education and advocacy about animal research.

S2A2.5

Communicating with Impact about Animal Research at your Institution

E. Maciejewski[1]
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Abstract
Pressure from animal rights activists, limited public awareness of the field of lab animal research, and a lack of established best communication practices have resulted in reluctance on the part of animal research institutions to communicate with the public and the media. Foundation for Biomedical Research director of communications Eva Maciejewski will present highlights of the Foundation's public opinion data on animal research. She will then delve into messaging strategies developed by the Foundation to establish a dialogue with the public about animal research. These messaging strategies include demonstrating the benefits of animal research for humans as well as animals, and rhetorical discussions about a future where biomedical research is conducted strictly with non-animal research methods. In this lecture Maciejewski will also discuss best practices in media relations related to animal research. As a connector between animal research communicators and the public, the news media is a powerful tool that must be handled methodically to convey the right message to the public at the right time. At the end of the lecture, participants will be empowered to develop their own public messaging about the animal research taking place at their institution.

S2B1.1

IVF: The Engine of Evolution in the Modern Embryology Lab

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Abstract
The production of mouse embryos at early developmental stages is crucial for the efficient management of transgenic mouse colonies and for maintaining research excellence. Therefore, it is necessary to have a robust and efficient embryo production pipeline. Approximately 10 years ago, the Charles River EU embryology platform refined its procedures for early development mouse embryo production. Previously, the pipeline relied on *in vivo* fertilization by mating males with hormone-primed females. However, this approach had several weaknesses. In contemporary embryology, in vitro fertilization (IVF) has emerged as a pivotal driver of key evolutionary advancements.

Through a retrospective analysis of 10 years of activity on our platform, we highlight the diverse applications and benefits of IVF and derived technologies in managing genetically modified mouse models. IVF allowed us to significantly reduce the time and resources required for generating, maintaining and securing genetically complex mouse colonies, adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement). We also drastically reduced the timelines for generating congenic lines and generation of recombinase edited alleles. Moreover, the integration of advanced techniques, such as laser-assisted IVF, has expanded the capabilities of traditional IVF.

This approach has led to the saving of thousands of work hours and mice. By showcasing the latest advancements and methodologies, we demonstrate that relevant technologies and appropriate staff training can allow faster and more reliable research while dramatically reducing the resources and animal number needed.

S2B1.2

Non-infectious and Infectious Disease Conditions of NSG and Other Severely Immunodeficient Mouse Strains

M. Hart[1]
[1]*IDEXX BioAnalytics, Columbia, United States*

Abstract
Mice with targeted mutations in multiple genes (NOD; Prkdc scid or *Rag1/Rag2*; and IL2 receptor gamma) lack mature T, B, and NK lymphocytes as well as hemolytic complement activity, and have diminished macrophage and dendritic cell function. Severely immunocompromised immune status renders these animals more susceptible to infection by many opportunistic organisms that rarely cause disease in immunocompetent mice. These animals thus pose many unique colony management, infectious disease prevention, and animal health monitoring challenges. This seminar will provide an overview of common infectious and non-infectious disease states found in NSG and other severely immunodeficient mouse strains. The targeted audience will be laboratory animal veterinarians, facility managers, and husbandry and research personnel.

S2B1.3

Balancing Animal Welfare and Disease Pathology in Cholestasis Studies

D. Zechner[1], G. Tang[1,2], W.-F. Nierath[1], E. Leitner[1], W. Xie[1], D. Revskij[1], N. Seume[1], X. Zhang[1,3], L. Ehlers[1] and B. Vollmar[1]
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Abstract
Cholestatic diseases such as primary biliary or sclerosing cholangitis are often investigated in mice after performing common bile duct ligation (cBDL). This study explored whether modifications to the cBDL model, such as partial bile duct ligation (pBDL) causes less distress to the animals, while allowing the investigation of pathological features.

Partial and total cholestasis were induced in mice, and animal well-being was evaluated using burrowing behavior, body weight, and a distress scoring system. To compare the pathological features between these models, plasma levels of liver enzymes, liver necrosis, fibrosis, and the expression of genes involved in bile acid synthesis (e.g., *Cyp7a, Cyp8b1*, and *Cyp27a*) were analyzed on day 14 post-intervention.

Mice subjected to pBDL exhibited a higher survival rate and significantly better well-being compared to cBDL mice. Both models allowed the study of localized liver damage, including necrosis, collagen I deposition, and immune cell infiltration (chloroacetate-positive cells). However, only cBDL mice showed markedly elevated liver enzyme levels, such as aspartate aminotransferase and alanine aminotransferase. Notably, *Cyp7a* and *Cyp8b1* expression was suppressed in both the ligated and unligated liver lobes of pBDL mice, suggesting that systemic mechanisms, in addition to local mechanisms, regulate these genes.

In conclusion, pBDL presents a less distressing alternative to cBDL, aligning better with animal welfare principles. While pBDL is less suited for examing systemic features of cholestasis, it provides unique opportunities for investigating novel mechanisms involved in cholestasis-induced gene expression.

S2B1.4

Improved Survival Rates for Swine Undergoing Myocardial Ischemia and Reperfusion Injury

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Abstract
Swine are commonly used in myocardial ischemia/reperfusion (I/R) injury studies, often with mortality rates of over 15% reported in the literature1,2,3. This study aimed to reduce mortality in a 60-minute swine I/R model through enhanced anesthetic management and perioperative monitoring. Female Yorkshire pigs (n = 68) underwent closed-chest cardiac ischemic injury via carotid access and dilatation balloon inflation in the left anterior descending artery (LAD) distal to the second diagonal branch. Pre-surgical oral medications included clopidogrel (300 mg loading dose, then 75 mg SID until surgery), aspirin (325 mg SID), and amiodarone (200 mg SID). Sedation was provided with an intramuscular injection of tiletamine/zolazepam (6 mg/kg) and induction with propofol as needed. Endotracheal intubation was performed and anesthesia maintained with isoflurane (0.5–3%) and a lidocaine constant rate infusion (20–40 mcg/kg/min) was provided. Monitoring included ECG, capnography, SpO2, digital mechanical ventilation, and direct blood pressure monitoring through the carotid access point. External defibrillation patches were secured to the animal. Perioperative support was provided through minimizing isoflurane inhalation during ischemia, continuous monitoring of vitals, defibrillation and emergency intervention (blood pressure support, magnesium chloride, and additional lidocaine) as needed. Uniquely, this study was performed by licensed veterinarians and veterinary technicians as opposed to scientists or other research personnel. Our mortality rate utilizing this protocol and personnel was 2.94% (2/68). 53 animals required additional drug intervention with 20 animals requiring defibrillation. Our results show that comprehensive monitoring and timely interventions can improve outcomes through reduced mortality. Veterinary involvement and direct oversight of procedures with reported high mortality rates may improve survival outcomes.

S2B2.1

Refining Animal Experiments: An Extensive Update of the Humane Endpoints Website

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[2]Animal Welfare Body Utrecht, Utrecht University, Utrecht, Netherlands

Abstract
A humane endpoint is defined as the earliest indicator in an animal experiment of (potential) pain and/or distress that can be used to avoid or limit pain and/or distress by taking actions. Taking actions does not necessarily mean the humane killing of the animal, but also includes conducting interventions to alleviate the stress- or painful experimental procedure or providing analgesics.

The widely used, publicly accessible Humane Endpoints website (https://www.humane-endpoints.info) has been developed to facilitate professionals who work with laboratory animals to define, recognize and implement humane endpoints in their work. The website and associated e-learning is not only suitable for education and training purposes, but also serves as a reference tool. The site currently contains extensive information on mice, rats and zebrafish.

The Humane Endpoints website is part of the 3Rs Research Tools Program, which is managed and hosted by the 3Rs Centre Utrecht. Currently, we are conducting a major update of the website, and we will present our plans. The aim is to improve the user experience by visual updates. Moreover, we are updating the content and complementing it with additional information.

In addition, we aim to enhance the international embedding of the Humane Endpoints website by seeking feedback from international experts and FELASA members. To ensure that it remains the go-to tool for professionals in the future to improve their knowledge

on humane endpoints and animal welfare, to refine their experiments and to improve the scientific quality of their research.

S2B2.2

Finding The Needle in The Haystack: Practical Diagnostic Approach To Unexpected Rodent Health Issues

M. Hart[1]
[1]IDEXX BioAnalytics, Columbia, United States

Abstract
Unexpected morbidity and mortality in contemporary rodent research colonies can significantly slow or completely halt research projects for affected investigators. Animal loss or confounded data generated from diseased animals can render experimental data difficult to interpret or unusable. In many cases these outcomes also impact multiple investigators sharing housing or workspace. Unexpected morbidity or mortality can be caused by, or related to, various factors including husbandry, environment, experimental manipulation, genetics, or infectious disease with most infectious agents being rare or opportunistic agents not typically found by routine animal health monitoring. Developing an appropriate and timely diagnostic approach for a sick animal or widespread illness/death within a rodent colony can help to mitigate the situation and resume research more rapidly. This presentation will outline best practice approaches for sample collection methods and techniques, sample handling guidelines, and expectations regarding next steps. This presentation will contain useful information for veterinarians, veterinary technicians, facility managers, and scientists.

S2B2.3

Supporting the LAS Surgeon to Succeed - Lessons from Healthcare about Training and Mentoring

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[3]Vetsalius, Lyon, France

Abstract
Procedures on animals should be carried out by competent persons (Directive 2010/63/EU, article 23).

After completing the modular introductory training, practical surgical training is then carried out within the research institute the 'on-the-job'. However, this training tends to focus on the particular surgical procedure required for that research model or experiment and results in a relatively narrow and technically-oriented focus.

This is in contrast to clinical surgery training, where the surgeon learns through a more holistic approach, providing the surgeon with a depth of knowledge and skill to address a variety of situations. During this time experts, mentors and peers are available to assist the learner's continued development and processes such as clinical audit assist in maintaining standards and refining techniques to optimise outcome.

In contrast, the researcher surgeon may lack access to the expert help that they need and resort to using online sources, which may or may not be peer reviewed, leading to misinformation, poor outcome and frustration. Having a suitably experienced surgical mentor to guide you can assist with planning, training, troubleshooting and quality assurance of the procedure, enabling smooth running of a surgical study.

This presentation examines how good quality surgical training could be organised within the LAS training framework, what we can learn from the healthcare setting, using technologies and how, e.g. having access to experienced mentors, can assist researchers to improve their procedures and adopt the most up-to-date methods, ensuring both the reproducibility of studies and safeguarding animal welfare.

S2B2.4

Can We Really Detect Pain in Laboratory Rodents (?) - Perspectives from Pain-Research

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Abstract
The most significant concern regarding animal research is the risk that experimental procedures may cause pain and suffering. Decreasing pain is crucial to maintain both experimental reliability and ethical integrity. But in order to prevent and decrease pain, we must be able to assess pain-related outcomes confidently, despite the obvious "language barrier" between rodent and man. A variety of paradigms and techniques have been developed, claiming to be able to detect pain-related changes, predominantly on behavioral readouts, in laboratory rodents. In the pain research field, the focus is on first creating models with pain-pathology that reflect the human condition, and secondly on assessing the pain-related mechanisms and phenotype as robustly as possible, to develop new therapeutic treatment avenues. Different approaches have been established to assess pain-related behavior as objectively as possible, initially focusing mainly on sensory stimuli, but in recent history also focusing increasingly more on natural behavior, like home-cage activity, climbing behavior, burrowing, facial grimacing or gait. Many outcomes may reflect relevant aspects of pain-related symptoms, but the sensitivity often depends on type of condition, timing of assessment, or are highly affected by animal related factors, like stress or fear of showing vulnerability. Detecting pain-related symptoms in rodents is possible but requires careful consideration and tailoring to the specific experimental settings. From the pain-research perspective, I will present and discuss the strengths and weaknesses of different assays or outcome measures that may be applicable to assessment of pain in the general laboratory animal setting.

S2C1.1

The Management of Ageing Laboratory Mice: A Challenging Resource

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[4]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

Abstract
There is a growing belief that drawing conclusions on treatments targeted to slow ageing based exclusively on lifespan data may be misleading. This flaw is due to a wide subset of age-sensitive phenotypes, such as muscle atrophy, cognitive decline, and age-related hearing loss (ARHL) that do not affect lifetime but would require refined and tailored husbandry and care. Inadequate acoustic environment, for example, impacts multiple organ functions leading to strain-dependent secondary changes including modifications in endocrine and cardiovascular function, sleep–wake cycle alterations, and many behavioural changes. To add a piece to this complex setting, we tracked age-, strain- and sex-dependent differences in the evolution of ARHL in CD1 and C57BL/6N mice up to 18 months of age derived from internal colonies to help identify ideal age and phenotype to start collecting valuable insights for developing a refined management strategy in elderly subjects. The interest in the role of non-transgenic geriatric animals is significantly increasing in ageing studies frequently stemming mouse models of disease from genetic/non-genetic manipulations affecting result translatability. Thus, this poses a serious question: when and how does ageing start to require a codified management path eventually becoming a procedure according to the law? Concerning ARHL, starting at the transition from the young adult (6 months) to the adult age (12 months) when, based on our data, the morpho-functional decline starts, it would be suggestable to acknowledge its impact on the biological aspects under investigation, to monitor the chronic background noise levels and minimize the daily routine sounds.

S2C1.2

Chemosensory Decay in Fish and Mouse: From Phenotyping to Husbandry Management

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[2]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy*
[3]*Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy*
[4]*Institute of Biochemistry and Cell Biology, National Research Council of Italy (IBBC-CNR/EMMA/INFRAFRONTIER/IMPC), c/o International Campus "A. Buzzati-Traverso", Monterotondo, Italy*

Abstract
The decrease in the ability to detect and discriminate odors and tastants in aged animals is generally not lethal but may greatly impact quality of life and behaviour. It may affect reproduction, nutrition, general health and well-being. Notably, the clinical management of elderly individuals is currently a key challenge of the animal facilities involved in ageing research. We tracked the age-related chemosensory decay in two evolutionary distant model species, both validated as ageing models: the teleost fish *Nothobranchius furzeri*, also known as the African turquoise killifish, and the laboratory mouse (*Mus musculus*). The two species possess also divergent biological features, being carnivorous and microsmatic, herbivorous and macrosmatic, fish and mice respectively. For both species, we selected outbred strains, MZM-0410 and CD-1 respectively, and identified timepoints representative of three life-stages (2, 6, 12 months for fish; 6, 12 and 18 months for mice). Species-specific behavioural tests were complemented by *ex vivo* analysis to confirm at morphological and molecular levels the behavioural observations. While we observed a drastic decline in chemosensory perception in elderly killifish, causing a drop in the food consumption and anorexia, we identified a decline in sniffing behavior and a change in taste perception in 18-months old mice, which showed less aversion to bitter substances and increased intake of saccharin. Altogether, our data contribute to better feature the biology of ageing in these two species. The outcomes of our study are useful to implement also the clinical score-sheet to be daily used in facilities where old animals are housed.

S2C1.3

Biological Adaptation to Sterile Environment Does Not Promote Adverse Phenotypes in Gnotobiotic Rodents

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Abstract
The gut microbiome supports digestion, immunity, and pathogen defense, but imbalances play a role in various disorders. Gnotobiotic animals, raised in sterile environments, enable precise studies on microbiome effects. These models include germ-free animals without microorganisms or those colonized with defined ones. In Germany, a debate persists about whether germ-free conditions represent a burden for the animals given the changes due to their sterile upbringing.

Germ-free animals resemble their colonized counterparts but adapt to sterile conditions. Gnotobiotic rodents display healthy behaviors such as grooming, nesting, and reproduction, with lower animal loss rates. While their gastrointestinal tracts show differences, including an enlarged cecum, issues like cecal torsion are rare. Immune system adjustments to reduced antigen

exposure, as well as cecal enlargement, are reversible through microbial colonization. Moreover, in our over 30-year experience, we observed no increased risk of harmful phenotypes in germ-free animals compared to colonized ones. Despite this, German authorities classify germ-free maintenance as a "procedure" requiring authorization, citing potential risk such as contamination, cecal torsion, or reproductive issues. However, evidence suggest that germ-free conditions reduce the risk of harmful phenotypes. Furthermore, high-efficiency barriers like isolators maintain gnotobiotic animals free from microbes over extended periods.

Gnotobiotic models are crucial for understanding microbial contributions to host health. Specialist knowledge for their maintenance is ensured through breeder, supplier and user registration under the EU Directive. In our opinion, there is no evidence that germ-free status burdens animals. Thus, their maintenance does not meet the criteria for project authorization under the directive.

S2C1.4

The Importance of Controlling Home Cage Relative Humidity for Animal Welfare and Study Reproducibility

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Abstract
In animal facilities, laboratory rodents are regularly housed under standardized conditions where environmental factors including lighting, temperature and relative humidity (RH) are controlled centrally by a conventional HVAC system. While many conditions can be amply controlled, these systems often inadequately regulate RH leading to fluctuating RH levels inside animal rooms depending on factors such as outside temperature, facility maintenance etc. Much research focusing on how external factors such as temperature affect animal welfare, behavior and physiology, has been published, but the effect of RH on these parameters remains largely unknown. The effect of fluctuating RH imposed on animals in home cages is thus broadly an ignored external factor that might impact study outcomes by introducing noise in resulting data.

Here we present findings from our studies investigating how RH impacts laboratory animals in home cages, and whether RH influences study outcomes and thus reproducibility of animal studies. Preliminary data indicate that in an atopic dermatitis mouse model, housing the animals at a stable RH level of 45% increases clinical outcomes by almost two-fold versus housing at RH 70%. Moreover, pilot results indicate that in a preference study set-up, certain mouse strains might prefer establishing nests at higher RH levels versus lower. These findings indicate that seasonal variation in RH levels inside animal facilities might contribute to the irreproducibility crisis. Furthermore, maintaining a stable RH levels inside home cages could contribute to the 3R principle by increasing refinement, and reducing the number of animals necessary in animal studies.

S2C1.5

Thermoneutral Environment Improves Mouse Welfare and Reduces Stress in Metabolic Cages

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Abstract
Metabolic cages [MCs] are often used to collect feces and urine samples. However, the housing of mice in MCs can be stressful, potentially affecting parameters of interest.

We compared our standard protocol for individual MC housing (4 days at 23°C, 3 days of permanent acclimatisation followed by 24h sampling) with MC housing at thermoneutrality (4 days at 30°C). C57BL6/N mice were implanted with telemetric transmitters to collect ECG, blood pressure, core body temperature, and activity data.

Single-housed mice in the MC at 23°C had lower core body temperatures and higher heart and respiratory rates. In contrast, mice housed in MCs at 30°C had lower heart and respiratory rates and a higher core body temperatures compared to mice housed individually in home cages at 23°C. Mice exhibited increased food consumption and weight loss when housed in MCs at 23°C, combined with significantly increased brown fat activity compared to mice in MCs at 30°C. Male mice of the MC23 group exhibited torpor-like (energy saving) behaviour. They also showed increased corticosterone levels.

Our study demonstrates that housing mice in MCs at 23°C has a significant impact on their physiology and welfare due to a substantial cold stress. MC housing at thermoneutrality (30°C) provides a simple solution to improve their welfare.

Keywords
Metabolic cage, Temperature Stress, Laboratory Mice, Refinement, Distress

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S2C1.6

Sentinel-free Soiled Bedding Testing Detects Opportunistic Bacteria Not Detected by Soiled Bedding Sentinels

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Abstract
Previous studies report challenges with SOB detection in soiled bedding sentinels (SBS) and colonies of immunocompetent mice. To investigate these observations and sentinel-free soiled bedding (SFSB) sampling for PCR as an alternative detection method, we monitored transmission of several SOB from positive cohorts to naïve immunocompetent and immunodeficient mice through a combination of direct and environmental sampling for culture and SFSB PCR. Three female 6–8-week-old BALB/c mice positive for beta-hemolytic *Streptococcus* group *B, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis* were cohoused with two naive CD1 or NCG mice per cage (n = 3 per strain in six cages). Mice were directly sampled by strain every 2–3 days for two-weeks. Cohoused groups were maintained for an additional three-month period to provide soiled bedding to CD1 Nude or CD1 sentinel cages and sampled monthly for PCR and culture. Soiled bedding was sampled via contact media (SFSB) monthly for PCR. Transmission and detection of segmented filamentous bacteria, *Enterobacter cloacae, Enterobacter hormaechei* and MuAstV1 were also monitored. SOB transmission from positive to negative cage cohorts did not occur rapidly or robustly. Fecal PCR estimated copy numbers suggested dynamic colonization that tapered below detection levels for several agents at later time points. SFSB outperformed PCR or culture of sentinel mice with limited or no transmission of SOB to all sentinel animals for all agents, reinforcing that transmission to sentinels does not occur efficiently when housed on soiled bedding. This study advances our understanding of SOB transmission and the advantages of SFSB to improve SOB detection.

S2C2.1

Developments on Pain Management for Zebrafish from Birth to Death

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Abstract
Zebrafish are used as a model in a wide variety of experimental fields and as such are subject to invasive procedures. Any tissue damage is likely to give rise to pain thus to improve welfare researchers and carers require a means of recognising and alleviating pain. Behavioural changes provide operational welfare indicators that can be assessed by the tank side. Typically, adult zebrafish reduce activity, space use and responsiveness to food and other stimuli after tissue damaging procedures such as fin clipping and tagging. Similar behavioural responses have been observed in <5 days post fertilisation zebrafish exposed to painful stimuli thus there is evidence of pain in young forms. Information from empirical studies can be used to inform the development of pain management protocols. Examples of laboratory procedures that result in exposure to painful treatment can be refined to include the administration of pain-relieving drugs. Key questions on what, how and when to administer analgesia will be explored. A variety of drugs, doses and administration routes have been identified that effectively reduce pain responses in both young and adult zebrafish. Implementing pain management to reduce pain thereby improving welfare is an important refinement in experiments using zebrafish. A recent FELASA working group report provides guidance on ways to provide pain management in a variety of common procedures and this report can inform implementation of analgesia in zebrafish experimentation.

S2C2.2

The three EEEs (Environmental Enrichment Endeavours)

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[2]*The University of Nottingham, Nottingham, United Kingdom*
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Abstract
The zebrafish research community has been debating the provision of environmental enrichment (EE). Reviews of published data reveal clear preference for social housing and natural environments by these fish. The overall aim for EE should be to provide enriched experiences but not by anthropomorphising choices, disregarding husbandry and welfare, or overruling data acquisition criteria. There are 5 deliverable life experiences that all research zebrafish, not under procedure, should experience. 1. Being together in a mixed sex shoal 2. Good lighting with night and day - preferably including fading in sunrise and fading out sunset 3. Live food to chase and promote nature hunting behaviours 4. A good clean consistent supply of water and 5. A natural substrate image (preferably gravel) below the tank. The question remains, do zebrafish benefit from physical tank based EE? We have undertaken a large scale EE study with 5 experimental tanks to address this question. The tanks were given enrichment and some had gravel floor images, while others had black floors. A variable was the presence or absence of a neighbouring shoal of fish visible to the experimental fish. The location of the fish in relationship to enrichment, floor images and neighbouring fish was assessed twice a day. Preferences were seen and assessed over several months. The enrichments were moved around and comparisons were made. This study has been able to decipher some key preferences and behaviours, which reveal insights into how we should and should not enrich zebrafish tanks.

S2C2.3

Updates from the Zebrafish Refinement Working Group

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Abstract
Based on the Swiss Culture of Care Group initiated by the Swiss 3R Competence Centre (3RCC) to promote and support the implementation of a 'Culture of Care' in Swiss institutions conducting animal research, a specialised Zebrafish Refinement Group was formed in 2023. This group is open to anyone working with zebrafish in Switzerland who is interested in refining experimental procedures and improving housing conditions within the framework of laboratory animal science.

The use of zebrafish in scientific research is increasing. With growing interest in this species as a model organism, expanding knowledge about their welfare and care has become increasingly important. Publications reflecting this heightened focus include a 2019 review on the use of anesthetics and analgesics, the 2020 FELASA Zebrafish: Housing and husbandry recommendations, the 2022 FELASA-AALAS guidelines for monitoring laboratory fish diseases and health, and the 2023 FELASA report on pain management in zebrafish (s. also https://felasa.eu/working-groups).

To meet these evolving needs, and recognising the close connection between a culture of care and the refinement of both experimental and husbandry practices, the Zebrafish Refinement Group aims to foster knowledge exchange and build expertise in zebrafish care for research purposes. Currently, three working groups are addressing key topics: monitoring of genetically modified zebrafish, anesthesia/analgesia/euthanasia, and housing/enrichment.

The background of the Zebrafish Refinement Group, along with the latest outcomes from its working groups, will be presented at the FELASA Meeting 2025.

S2C2.4

Risk Assessment, Pathogen Reservoirs, and Pathogen Transmission in Zebrafish Facilities

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Abstract
Biosecurity and colony health management are critically important for the protection of animal health and welfare, the validity of experimental data, and personnel from zoonotic disease. Risks to zebrafish facilities can include introducing infectious agents via importation of new zebrafish lines, the use of live feeds, the use of contaminated biological materials, shared resources and equipment, or proximity to other aquatic organisms or systems. Many tools are available to mitigate biosecurity risks, including the use of approved vendor lists, colony-specific exclusion lists, quarantine, routine colony health surveillance, diagnostic investigation of morbidity and mortality, biosecure husbandry practices, and management of work and material flow. This presentation will focus on the important questions for assessing the risks associated with infectious agents, and the steps that can be taken to exclude infectious agents as well as to minimize the transmission and impact of infectious agents that are already present in zebrafish colonies.

S2C3.1

Life Course Husbandry for Zebrafish

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Abstract
The life course of the zebrafish can be divided into 5 stages; embryo, larval, juvenile, adult and aged fish. Standard rearing protocols to reach breeding adulthood take approximately 3–4 months. This can be variable due to factors such as stocking density, tank size and nutritional availability. At this speed of development, with our in-house parameters, fish routinely show aged phenotypes from 30 months. Speeding up the time to adulthood is possible and an important tool for establishing new GAA lines, particularly when multiple alleles are required for interbreeding over several generations. Research often has tight deadlines so there is a push to get results. This driver makes quick rearing times an essential research aim, which zebrafish facilities should reserve for specific programmes of work rather than general stock maintenance. Raising adults as young as possible does have consequences, significantly reducing their longevity, fecundity as well as inducing premature frailty, factors that researchers may not need to consider. It seems that these methods are now so widespread that the consensus is that zebrafish are aged by 18 months. Over time, a 1.5-year turnover will significantly impact the 3Rs, creating more fish than necessary. There may also be long-term consequences having these accelerated methods as standard practice. This little fish is robust and resilient, but it still requires careful husbandry considerations for its welfare and optimal life course potential. Let us not push it too hard.

S2C3.2

The Core Facility Concept of the Zebrafish Core Facility at Karolinska Institutet

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Abstract
The zebrafish core facility at Karolinska Institutet is a service hub for more than 60 internal and external research groups that use zebrafish in basic, biomedical and translational research.

Animal welfare and standardization of husbandry procedures and experimental pipelines as well as stringent quality control represent the central pillars of our efforts to deliver reliable and reproducible scientific data.

In this presentation, we would like to share details of our facility design, our core facility concept, our animal welfare measures, and how we facilitate education, collaboration, and harmonization between zebrafish facilities in the Nordic countries.

The facility is divided into physically separated epidemiological units and run by a team of seven staff including animal caretakers, technical staff, and research specialists. Together with our designated veterinarian, the facility is run based on an elaborate biosafety plan including directed and controlled movement of staff, animals, equipment, and consumables. Regular health checks are done on animals, biofilm, and feed according to the FELASA-AALAS guidelines.

In order to maximize standardization and animal welfare, all husbandry and animal deliveries are solely done by staff, users have only access to terminal procedure rooms. All procedures follow SOPs based on scientific evidence and in-house verification.

The core facility has also taken a leading role in establishing a national laboratory animal science course specifically for zebrafish and organizes an annual zebrafish husbandry course for animal caretakers. Moreover, we have recently founded a Nordic Zebrafish Community to facilitate education, collaboration and harmonization between zebrafish labs of the Nordic countries.

S2C3.3

Advancing Zebrafish Welfare through Innovative Breeding Strategies and Health Monitoring

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Abstract
Zebrafish have emerged as a key model organism in biomedical and environmental research. However, maintaining their health and well-being in laboratory settings remains a significant challenge, particularly regarding their environmental, nutritional, and reproductive needs. Recognizing the critical role of effective breeding strategies in zebrafish care, we have developed and implemented innovative breeding strategies that optimize reproductive outcomes while enhancing fish welfare. To support welfare assessment, we have engineered an interface for recording and tracking sick animals, enabling precise and efficient health monitoring. Combined with novel environmental enrichment strategies, these methods represent a significant step forward in zebrafish husbandry. Our presentation will detail these advancements, highlighting their impact on zebrafish welfare and the reproducibility of scientific outcomes. This work underscores the importance of tailored husbandry solutions in laboratory fish care and reaffirms our commitment to the 3Rs (Reduction, Refinement, and Replacement) in animal research, promoting improved welfare and more efficient use of research animals.

S2C3.4

Optimizing the Husbandry of the African turquoise Killifish: A Step Forward in the Standardization

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Abstract
Recently, the African turquoise killifish *Nothobranchius furzeri* has emerged as an important model for studying vertebrate biology. This annual fish lives in seasonal ponds in southeastern Africa and has a median lifespan of only 3–7 months, an adaptation to the ephemeral environment in which it lives, making it the shortest-lived vertebrate that can be bred in captivity. Despite its short lifespan, *N. furzeri* exhibits typical age-related traits and diseases, making it a valuable model for aging research. The management of large killifish colonies under standardized conditions presents challenges in terms of animal welfare, data repeatability and translatability. Our goal is to develop an optimized protocol routinely applicable to the colony management that can support researchers in the husbandry of this species. Starting from a review of existing protocols, we created detailed guidelines for egg incubation, hatching, daily care of juvenile and adult fish, feeding and breeding. To reduce contamination and variability, we standardized the diapause period using only humic acid. To improve hatching rates, we recommend using low temperatures for the incubation stuff. We refined the feeding routine for both juvenile and adult fish by feeding them three times a day to avoid delays in sexual maturity and by establishing a precise weaning technique and the amount of frozen bloodworms to be fed. For breeding, we recommend spawning once a week. We believe that improved housing and husbandry conditions could enhance killifish welfare and contribute to the development of breeding guidelines for this species.

S2C4.1

AALAS-FELASA Pig Training and Conditioning for Research Working Group: Overview and Current State

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[4]*University of Copenhagen, Copenhagen, Denmark*
[5]*Washington University, St. Louis, United States*
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Abstract
Pigs are an important species in research, used as models to further knowledge in disease pathophysiology, pharmacokinetics, toxicology, surgical techniques, biomedical device development and xenotransplantation. There is ample evidence showing that reducing stress for research animals benefits animal welfare and research outcomes. The goal of the AALAS-FELASA working group is to develop best practices, including use of classical and operant conditioning and environmental enrichment to work cooperatively with pigs in research. This presentation will provide an overview of the working group, as well as present results from a survey conducted in 2024 to assess the current state of training and conditioning with research pigs, and better understand the current barriers and ongoing challenges. There were 168 responses across North America and Europe. Most respondents worked with pigs as a veterinarian (54%), researcher (44%), and/or animal care/husbandry personnel (41%). About 80% of participants had received some education on large animal training, behavior, or conditioning during their careers. Habituation (66%) and positive reinforcement training (69%) were used often with pigs, but mainly for long-term studies (75%). The benefits of training and

conditioning were seen as less stress for the animals (99%), animals are easier to work with (98%), improved job satisfaction (92%), improved study data (79%), and cognitive benefits for the pigs (77%). The primary challenges were seen as time (76%) and human/staff resources (69%). To implement more training and conditioning with research pigs, participants were interested in more live workshops and discussions (61%), on-demand training (56%), and in-person demonstrations (51%).

S2C4.3

Environmental Impacts on Pig Welfare: Enrichment and Social Environment

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Abstract
The purpose of environmental enrichment (EE) is to enable pigs to express their natural behaviors, stimulating their visual, somatosensory, and olfactory systems. Pigs have an inherent tendency to socialize, explore, root and chew. The EE provided to laboratory pigs should address not only physical and occupational aspects but also nutritional and social elements. When choosing materials to enhance pig welfare, the enrichment should be safe, clean, and accessible in sufficient amounts. Materials are categorized as optimal (e.g., bedding), suboptimal (e.g., chewing toys) and of marginal interest (e.g., balls). Since most knowledge about EE for pigs comes from conventions farms, various factors must be considered when transferring to lab settings, such as health profiles, physiological status, and naturally occurring or induced pathological stages. The selection of enrichment materials should account for the animals' health status and the procedures they will undergo, while maintaining study design consistency. For example, straw may be an ideal EE material, but may not be suitable for certain controlled health colonies or post-surgery cage setups. Social interactions, both intraspecific and interspecific, are crucial for psychological well-being. If isolation is necessary for experimental reasons, maintaining some form of social connection, such as adjoining pens or shared spaces, is advised. Additionally, regular interaction with staff serves as beneficial enrichment, helping pigs adapt to handling and reducing stress. In conclusion, it is important to develop and follow a standardized enrichment plan, which includes rotating materials, providing adequate nutritional enrichment, and incorporating animal training to enhance the psychological well-being of pigs.

S2C4.4

Principles of Learning and Practical Application of Training with Research Pigs

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Abstract
Pigs are intelligent animals; they are easy to train and they learn all the time. By implementing training of the pigs, we can provide better welfare throughout their entire lives, improve data quality and enhance animal caretaker job satisfaction. According to the EU directive 2010/63/EU, "establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project". Additionally, training the animals can affect the severity category of an experiment.

All animal training builds on the principles of classical and operant conditioning, but additionally it is important to understand how to set the animal up for success and how to get the behaviour. Pigs can be trained to voluntarily participate in husbandry procedures, medical procedures and experimental procedures. This talk will give video examples of trained behaviours and how to set up the training and the effect on animal welfare will be discussed.

Training animals demands resources; however, depending on the study in question, the time invested in training the pigs may often be recuperated as procedures can be carried out smoother and with fewer people. Even though training not always seems feasible, training of the pigs should be done whenever possible to increase animal welfare and to gain a better understanding of the possibilities to enhance pig welfare through training.

S2C4.5

Enhancing Welfare and Study Outcomes through Positive Reinforcement Training in Göttingen Minipigs

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Abstract
At our research facility, we prioritize the welfare and effective handling of Göttingen minipigs and Swedish landrace pigs to improve study outcomes. Our training program, initiated upon the animals' arrival, focuses on positive reinforcement using treats. This approach reduces stress and fear, fostering curiosity and calmness in the pigs, which facilitates easier handling during studies.

We have developed a training schedule with a scoring system to assess and improve each pig's progress. Initial training includes frequent sessions that decrease in frequency, focusing on acclimating pigs to various handling techniques and study-specific tasks like ECG band fitting and oral dosing.

A central feature of our methodology is the use of central venous catheters (CVCs) for blood sampling, minimizing repeated needle sticks and stress. To extend catheter functionality, we employ sterile placement, enhanced care protocols, and use a "dummy" for staff training. Innovations such as start-stop flushing and backflow valves have extended catheter lifespan to 8–10 weeks.

Our novel oral dosing method uses jam to deliver medication, eliminating the need for restrictive handling. This technique is well-received by pigs, promoting voluntary cooperation and improving animal and staff welfare.

These practices reflect our commitment to advancing animal welfare and optimizing research outcomes, demonstrating significant improvements in procedural efficiency and study integrity.

S2C4.6

Culture of Care at the Vendor: Little by Little, a Little Becomes a Lot

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Abstract
Ensuring a good Culture of Care and advancing its level is an ongoing process. Several factors, such as mindset, competence, and communication, are key in this work. At Ellegaard Göttingen Minipigs, promoting a proactive mindset is a priority, starting from onboarding. Subsequently, continued focus on this is ensured, e.g. through themed days and activities hosted by the animal welfare body. Rule compliance is essential but promoting a culture of challenge of existing norms and standards is also necessary and is fostered through various measures. Measures include encouraging animal care staff participation in enrichment strategies and implementation, also, systems to ensure that all voices are heard are in place. Competence is ensured through training and retraining as necessary. This is extended to include both personnel directly and indirectly involved in animal care and welfare. Examples include educating animal care staff on options for refinement, such as minimally invasive sampling, habituation, and positive reinforcement training, and providing thorough onboarding to new members of the animal welfare body. Open and transparent communication on the use and effect of the work with laboratory animals supports employee compassion satisfaction by aiding in social support and acknowledgement of their invaluable contributions to science, ultimately benefitting humans and animals alike.

S2D1.1

Implementing an Effective and Realistic Culture of Care in Europe

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Abstract
The implementation of a culture of care in research animal facilities is crucial for ensuring not only the welfare of animals, the integrity of scientific research and also the wellbeing of all personnel involved. A culture of care is defined as a collective commitment to the ethical treatment of animals, encompassing attitudes, behaviors, and practices that prioritize animal welfare and responsible research.

This presentation aims to provide through real examples a comprehensive overview of effective and realistic strategies for fostering a culture of care within European research animal facilities. The presented examples will come from observations made during AAALAC International site visits performed lately in Europe.

We will explore the impact of Directive 2010/63/EU, which sets the standards for the protection of animals used for scientific purposes, and how it has shaped the practices and policies in research institutions.

Key components such as staff training, ethical review processes, continuous improvement, and actual implementation of the 3Rs will be discussed. The role of Animal Welfare Bodies (AWBs) and Ethics Committees in promoting and overseeing the culture of care will be highlighted, emphasizing their importance in ensuring compliance and continuous improvement.

Additionally, we will delve into the notion of teamwork and the involvement of all stakeholders, from researchers to animal care staff, in creating a supportive and collaborative environment. Attendees will gain insights into best practices and innovative approaches that have been successfully implemented across various institutions.

S2D1.2

A North American Perspective on Interpretation and Implementation of a Culture of Care

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Abstract
Establishing a Culture of Care in the context of producing and working with animals in biomedical research describes the culture within institutions to strive for continuous improvement in animal care and welfare, care and welfare of personnel involved in caring for and working with animals, ensuring scientific quality and integrity, and providing for openness and transparency inside and outside the workplace. Using this inclusive definition creates an opportunity for everyone at a given institution to relate and contribute to change. Regardless of an individual's role and perspective within the institution, be it reduction of overall institutional risk; improving consideration of animal welfare and practices surrounding their care; answering fundamental scientific questions or developing new therapies for patients; assuring regulatory compliance; securing retention of highly qualified staff and scientists; reducing bite and exposure risks for personnel handling animals; and others, cultivating and sustaining an expectation for a good Culture of Care is a shared responsibility. This session will provide specific examples of regional strategies in these four areas including developing robust outcomes-focused behaviour management programs for rats; implementation of non-aversive handling practices for mice and rats; provision of in-house compassion science programs for vivarium personnel; use of objective validation studies to improve experimental design and thus data quality, and exemplars of the U.S. Animal Research Openness (USARO) initiative.

S2D1.3

Promoting a Culture of Care: Challenges and Opportunities in Asia-Pacific Laboratory Animal Facilities

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Abstract
A "culture of care" is being increasingly acknowledged in the field of Comparative Medicine in the Asia-Pacific region. This concept not only emphasizes the ethical treatment of animals, including the application of good animal welfare practices and the 3Rs (Replacement, Reduction, Refinement), but also extends to include the well-being of the personnel working within these facilities as well as research integrity, reproducibility, institutional transparency and societal openness.

Covering a broad geographic and culturally diverse region, where attitudes towards animals and the importance of animal welfare are rapidly changing, understanding the specific regional challenges and opportunities is key to the successful promotion of the 'Culture of Care' in Asia-Pacific.

We will explore:

The role of the Institutional Animal Care and Use Committees (IACUCs) and their importance in promoting the "culture of care" by ensuring adherence to ethical guidelines; the instilling a continuous improvement mindset; the provision of adequate resources; and the training and education of personnel.

How empowerment of staff and their active involvement in decision making, and the ability to raise suggestions or concerns are critical to fostering a culture that not only respects animal welfare, but also contributing to personnel well-being.

How a good "culture of care" promotes openness and a culture of respect and integrity that will inevitably lead to better research reproducibility and outcomes.

Examples of how aspects of "culture of care" have been successfully implemented as well as further opportunities for institutions, regional societies and international accreditation bodies such as AAALAC International will also be discussed.

S2D1.4

The International Culture of Care Network: Sharing and Promotion of Culture of Care Practices

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Abstract
In this presentation the International Culture of Care Network will be presented. The idea to establish the network was proposed at the FELASA Congress in Brussels in June 2016 and was thereafter established in September 2016. There are currently 57 individual members from 14 countries in the Network. The primary role of the network is to share and publish examples of activities fostering a Culture of Care which make a difference in terms of improved animal welfare and human wellbeing. Working with a Culture of Care is abstract and is linked to ideas, concepts, local culture on several levels, both within companies, cultures, and societies. This means that helping and inspiring each other get to be an advantage in the work with and implementation of a culture of care.

This presentation will go through the history of the network, what the network has worked with and the purpose and advantages in having such a network. The network is always open for more interested and active members. If you are interested in learning more, please contact the Network at CultureOfCare Network@gmail.com.

S2E1.1

Knowing Better, Doing Better in Experimental Design Education

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[2]*University of Bologna, Bologna, Italy*
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[6]*Janssen Pharmaceutica NV, Beerse, Belgium*

Abstract
Research practices are arguably too often constrained by the somewhat narrow and traditional ways in which we evaluate quality, support careers, and recognise contributions made to research. Can positive practices be implemented? Within this general framework, and in the spirit of this session, this presentation focusses on education in Experimental Design and the output from the FELASA Experimental Design Working Group.

The group considered the current provision of Experimental Design teaching and how it might be improved and harmonised across the Laboratory Animal Science community. The output, whose salient points are addressed in the presentation, provides specific, practical and trialled recommendations. They include Learning Outcomes principally aimed at early career researchers, example of content, scenarios and timetable and the ideal skill set for tutors.

S2E1.2

Every Mouse Counts! The Validation of the Experimental Unit in the Focus of 3R

M.A. Vogt[1], I. Schniewind[1], T.M. Lohr[1], S.K. Balcerzak[1], P. Follert[1] and S. Chourbaji[1]
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Abstract
To justify animal experiments, modern research relies on advanced methodologies, with statistical techniques playing a key role in reducing animal use. Often overlooked, statistics help minimize subject numbers through biometrical predictions and, importantly, correct identification of the experimental unit (EU).

The EU is defined as the smallest unit that can be treated differently within an experiment. Proper EU implementation can increase animal numbers when 'treatment' refers to housing conditions, as the **cage**, regardless of the number of animals, is the EU. Approaches such as ignoring social behavior, testing a 'cage representative', or using the mean of the cage data can result in surplus animals. Alternatively, testing all animals within a cage but using only the cage mean could address this.

To evaluate these approaches—single housing, 'cage representative', 'cage mean', or testing all individuals within a cage — we assessed inbred **C57BL/6NRj** and outbred **SWISS** mice of both sexes. We examined animal welfare through behavior and physiology, including activity, anxiety, nociception, sensorimotor skills,

exploration, and corticosterone levels. Social hierarchy was evaluated weekly to assess correlations with behavior.

Our results indicate that housing condition did not affect outcomes. Data quality, assessed by **coefficient of variation**, depended on the parameter measured, not housing condition. Hierarchical ranks remained stable and did not correlate with behavior.

Using the EU as a 'mean' or 'representative' of the cage produces similar results to individual analysis, still avoiding single-housing and therefore prioritizing social needs and improving animal welfare, with greater respect for the individual lives of the mice.

S2E1.3

We Need to Talk about Sexes - Addressing the Prevalence of Single-Sex Animal Studies

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[2]*University of Manchester, Manchester, United Kingdom*

Abstract
The prevalence of single-sex studies in biomedical research raises significant ethical, scientific, and methodological concerns. Although funders are pressing for both-sex studies, discussion with researchers reveals a lack of confidence in undertaking these, and a need to talk about the difficulties. The male bias in the biomedical literature is often reported to result from the belief that oestrous cycles cause higher variability in females, despite not supported by evidence (1). When research favours using females, it is typically to avoid within-cage aggression in males, which is better addressed through good husbandry practices.

Using only one sex for studies that are not sex-specific may lose important information on how sex influences disease development, as well as the response to – or side effects of – candidate therapies. It can also lead to unnecessary extra breeding, and culling of animals of the unused sex. This wasteful approach undermines the Culture of Care, giving animal care staff a higher work and emotional-burden, contributing to burnout and compassion fatigue.

Teaching on experimental design should address how to deal with aggression and variability, and provide researchers with confidence in using both sexes. For example, it can show that a sample size calculated for a single sex study can be adapted to include both males and females, with little to no loss of statistical power, and that factorial designs can detect sex-treatment interactions (2). Such designs allow for more robust and generalizable results, better use of resources, and the furthering of the 3Rs and responsible research practices.

S2E1.4

True Story Telling: (Pre)Registration in Practice

J. Menon[1]
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Abstract
The increased emphasis on improving animal research quality has driven the development of new methods. This presentation will focus on one of them: preregistration (the recording of a research protocol before the study starts), and will address three aspects:

Advantages: Preregistration increases transparency and reproducibility by openly sharing key methodological details (e.g., hypotheses, study design, and analysis plans) in advance, encouraging early methodological planning and reducing reporting biases and questionable research practices (e.g., selective outcomes reporting, HARKing, and p-hacking). Preregistration also helps prevent involuntary duplication and publication bias by displaying all studies, including unpublished ones, and allowing protocols to be linked to related data.

Mechanisms: the preregistration process will be illustrated using Preclinicaltrials.eu the first registry dedicated to the preregistration of animal studies, as an example. Launched in 2017, this non-profit, web-based registry provides free, universally accessible, and anonymous registrations. It offers three key features to provide a secure, flexible and user-friendly process. First, researchers can opt for an embargo that keeps protocols confidential. Second, researchers can amend protocols through track-changes that create a transparent audit trail. Third, researchers can preregister easily through fast exports that use their ethical documentation.

Concerns: Finally, I will address worries that are voiced by some stakeholders in using the preregistration process, present evidence of its effectiveness and provide an overview of its current adoption. Join us to discover how preregistration can strengthen the future of animal research.

S2E2.1

Home Cage Monitoring - Developments and Opportunities

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Abstract
Home-Cage Monitoring, by many different technical platforms, is greatly contributing to our understanding of the behaviour and activity of a number of different mouse strains over long periods of time, including times where normal observations by researchers are rarely recorded (such as in the dark periods). In recent years, advances in technology have delivered better quality scientific data alongside improvements in animal welfare.

In this workshop, we will highlight important new developments in automated welfare and phenotypic analysis for laboratory animals and the role they play in reducing subjectivity and enhancing the relevance and translatability of animal studies. In particular, we discuss the opportunities and potential impact of Home Cage Monitoring (HCM) systems on reducing both the numbers of animals used in research (Reduction) and their discomfort (Refinement). We also address the significant logistical, cultural and resource challenges that the research animal community must overcome to attract and collaborate effectively with informaticians and data scientists to realise these ambitions.

Recent advances in artificial intelligence (AI) and, in particular, machine learning (ML) technologies present significant

opportunities to drive substantial progress in laboratory animal science. However, to realise this potential, the animal research community must radically change the way in which it generates, annotates and shares large datasets to enable and foster interdisciplinary collaboration required.

S2E2.2

Applying New Home Cage Monitoring Technologies to Welfare Assessment

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Abstract
In nature, mice are the epitome of a prey animal. High vigilance, digging, and hiding are just some of the behaviors used to avoid predators. One should be aware that the natural behavioral repertoire is also preserved in laboratory mice. It is therefore not surprising that the animals become easily stressed when they are picked up and taken from their safe environment. Under such conditions it would be unfavorable to expose one's vulnerable side to a potential attacker, and thus it is sometimes difficult to determine poor welfare outside of the safe home environment. New techniques (video tracking, RFID, bioacoustics) allow long-term observations in the home cage to be performed at increasingly high levels. Such techniques also allow to monitor the well-being within the home cage as well to conduct behavioral research without unnecessarily stressing the animals. Being able to test within the home cage however, will add to the importance of improving housing conditions as laboratory mice spend almost their entire lives in small barren cages. Under such constraint housing conditions mice are usually unable to show their full repertoire of natural behaviors. Therefore, refinement of cage size and design must be considered continuously for better welfare and for better science.

S2E2.3

Smarter Monitoring: Data Science Innovations in Automated Home Cage Systems

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Abstract
Advancing welfare assessment in laboratory animal science demands precision, objectivity, and reproducibility. These requirements become even more challenging with the advent of automated home cage systems, which generate time-dependent, high-resolution data.

This presentation explores innovative data science methodologies applicable to large datasets for severity and welfare assessment. We discuss the application of structural equation modeling, logistic regression, and sensor fusion as foundational elements of an analytical pipeline aimed at refining objective severity classification and improving humane endpoint identification. Building on frameworks such as Relative Severity Assessment (RELSA), we present multidimensional quantitative approaches that integrate physiological, behavioral, and clinical indicators, effectively synthesizing home cage systems' rich, multifaceted data. These methodologies balance single-animal severity assessment and procedural robustness amidst the inherent variability of time-resolved biological data. Additionally, we demonstrate near-real-time, retrospective, and prospective quantitative severity assessments, highlighting the technical and analytical expertise required to implement such analyses.

While automated systems with continuous data acquisition capabilities reduce observational bias, increase assessment frequency, and minimize disruption to animal behavior, they also introduce significant technological and analytical complexities. Despite these challenges, the translational potential of these data-driven methodologies extends beyond laboratory settings into preclinical and clinical research, offering scalable solutions for welfare monitoring in human contexts. By bridging data science and laboratory animal welfare, this work advances more humane, scientifically rigorous, and ethically sound practices, establishing a foundation for future innovations in automated monitoring technologies.

S2E2.4

iMouse System - A Visual Method for Standardized Digital Data Acquisition Reduces Severity Levels

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[4]*Faculty of Health Sciences Brandenburg, Brandenburg Medical School Theodor Fontane, Potsdam, Germany*
[5]*Department of Gastroenterology, Center for Translational Medicine, University Hospital Brandenburg, Brandenburg Medical School Theodor Fontane, Potsdam, Germany*

Abstract
In translational research, using experimental animals remains standard for assessing the effectiveness of potential therapeutics. At the same time, minimizing the impact on the well-being of the animals regarding the 3R is mandatory. To fulfil this goal and therefore evaluate the severity level, animals must be inspected several times a day. It is noted that these visual assessments disrupt the animals during their resting periods, resulting in elevated stress levels and consequently affect the results of scientific studies. We examined the feasibility of implementing a digital monitoring system (iMouse) in a translational study conducted within home-cages. Our objective was to reduce or replace manual visual inspections during experiments and to examine whether digitally available data from this study can be used to train an algorithm capable of distinguishing between activities. We successfully demonstrated the feasibility of integrate the system into the existing IVCs and established remote access to

the overserved home cages. Accordingly, digital surveillance of the experimental animal reduces their stress level. Furthermore, the digitally acquired data out of the home cages proved instrumental in training algorithms capable of analysing e.g. the long-term drinking behaviour of the animals. In summary, our work has yielded an integrated, retrofittable, and modular system that serves two critical criteria for the 3R. Firstly, it reduces the severity level of the animal by executing visual inspections. Secondly, it refines the traceability and transparency of animal-based research studies. The standardized iMouse system enables the analysis of data sets and the generation of new digital biomarkers.

S2E2.5

Early Biomarkers Detection in Neurodegenerative and Diabetes Models Via Continuous Home Cage Monitoring

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Abstract
Accurate monitoring of disease progression in animal models is essential for understanding pathophysiology and developing therapies, especially in the early phases. Recent studies indicate that novel methods, such as continuous digital biomarkers, can identify the presymptomatic phase of specific pathologies (1–2). We employed a high-throughput home cage monitoring system to identify early predictions of disease onset in amyotrophic lateral sclerosis (ALS) and diabetes mouse models.

In two SOD1G93A ALS mouse models (slow and fast-progressing), locomotor activity patterns became increasingly irregular, particularly during the daytime. These irregularities were quantitatively assessed using the Regularity Disruption Index (RDI), which correlated with declines in neuromuscular function and body weight loss. Additionally, distinct differences in voluntary running wheel activities were observed between models. Access to running wheels accelerated the onset of disease symptoms in the fast-progressing ALS model.

In diabetic mouse models, continuous monitoring of the urination index facilitated the identification of polyuria, a key symptom of diabetes associated with elevated blood glucose levels. This biomarker enabled non-invasive, continuous evaluation of diabetes onset, progression, and severity within the home cage environment. This methodology minimizes the necessity for invasive blood glucose measurements, improving animal welfare and yielding more reliable data on urination patterns.

Overall, 24/7 digital biomarker monitoring in a high-throughput home cage system significantly advances disease modeling by allowing early biomarker detection under stress-free conditions, thereby enhancing animal welfare and data reliability.

S2E3.1

3Rs Implementation in Fundamental Research – a Broad Perspective

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Abstract
The 3Rs Principles are the methodological backbone of contemporary animal research, with important ethical implications. Therefore, despite the fact that Russell and Burch proposed the 3Rs about 70 years ago, their idea is still valid today and supported by the current EU legislation in its practical implementation. However, a complete understanding and application of the Principles is still finding some resistance among the scientific community, and this situation detracts from the impact Russell and Burch's idea can have on experimental science. In this contribution I will discuss some of the possible reasons why sometimes the 3Rs are not implemented as fully as they could be. In particular, "the culture of others", a certain methodological inertia and failing to grasp the dynamical aspects of the Principles will be presented as possible factors. Furthermore where the Animal Welafare Body has to evaluate project proposals before sending them to the competent authority for autorisation, such is the case in Italy, the cultural role of the AWB to expand the culture of the 3Rs Principles among the researchers' community can be penalised. Possible ways to overcome these obstacles will be presented.

S2E3.2

3Rs Implementation in Neurosciences

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Abstract
The 3Rs Principles consist in Replacement, Reduction and Refinement.

How can they be applied in Neurosciences? Each R will be reviewed through examples to address whether or not they can be applied in a satisfactory manner and how this could be improved.

Replacement in Neurosciences might seem currently difficult when you want to study brain functions, while Reduction is easy but may raise reproductibility issues. Refinement is currently applied. Using the example of my research on animal models of addiction and other researches, perspectives and limitations will be discussed.

S2E3.3

Challenges of Applying 3Rs in the Field of Immunology

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Abstract
Since the ´60s, the 3Rs principles have been recognized by the scientific community as the cornerstone of ethical research in animal experimentation (*Russell and Burch., 1959*). For instance, the application of the 3Rs in the field of toxicology has resulted in a reduction in animal usage thanks to the development of *in vitro* systems and protocols that offered alternatives to the use of animals (*Hamburg., 2011*). This has been reinforced by the approval of some programs at the European level intended to reduce the usage of animal testing for chemical safety. However, the application of 3R methods in other biomedical areas has faced significant challenges. Immunology is a field heavily dependent on animal models, and it is one of the top research fields in terms of the number of animals used. The reasons for this situation are multiple. Amongst them, the complexity of the disease models, the presence of new pathogens such as the coronavirus, and the development of novel therapeutics in a very dynamic area of science are some of the reasons behind the poor application of 3R methods in this field. During the presentation, we will discuss different initiatives intended to promote the application of 3R methods in the field of immunology, such as the generation of computational tools allowing for robust simulations of immune responses or the generation of repositories that increase the accessibility of the data by enabling the storage of management of data sets in the immunology field.

S2E4.1

To Err Is Human: When Animal Work is a Source of Distraction

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Abstract
In research, the personnel that interact with animal models (e.g., animal care staff, veterinary professionals, and research team members) often change throughout the experimental and resting/housing periods until the end of study. One of the most common interactions with research animal species is between animal handler and animal at times of cage change, experimental manipulation, and physical examination. It is well established that these types of interactions, while necessary, are also a source of stress for the animals. An improved understanding of these influences of handlers on animal responses will be critical to consider in terms of research findings and general interpretations related to reproducibility in biomedical models of human disease. This talk will provide background and consideration for how to account for one of the greatest variables in research – the personnel themselves.

S2E4.2

Human-Mouse and Mouse-Mouse Interactions in Preclinical Research

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Abstract
Preclinical researchers have paid very little attention to the housing and testing environments of their research subjects. Interactions between humans and mice, and amongst mice themselves, have been shown to produce marked effects on, and thus represent confounds of, preclinical experiments. A number of examples relevant to pain will be discussed.

S2E4.3

Of Mice and Men: How to Deal with Heterogeneity in Animal Research

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Abstract
As in other fields of research, poor reproducibility in animal research has mainly been attributed to Questionable Research Practices (QRPs, e.g. selective reporting, post-hoc reasoning, p-hacking). However, the diagnosis "poor reproducibility" itself is often based on the questionable assumption of treatment effects that are fixed across time and context and can be classified into true and false using null hypothesis significance testing. Rather than being "furry test tubes", animals are plastic and their responses may vary depending on genotype (strain, sex), age, developmental history, and context (including animal-animal and human-animal interactions) whenever an effect is being measured. Such variation introduces heterogeneity to the results of independent replicate experiments, even in the absence of QRPs. But how should researchers deal with such heterogeneity when designing, conducting, and analysing animal experiments? The answer is: it depends on the aim of the experiment. Is the aim to explore a specific mechanism in an abstract animal model system (proof-of-principle)? To formally test a specific hypothesis (validation)? To investigate a reaction norm (treatment x context interaction)? Or to firmly establish a treatment effect before taking the treatment to a clinical trial (preclinical trial)? I will discuss the implications that such different study aims have for selecting the study population, designing the experiment, and analysing the data, offering some basic rules of thumb.

S2G1.1

Challenges in Maintaining Microbial Status of Rodents in Specific Pathogen Free and Conventional Housing

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Abstract
Routine microbiology, virology and parasitology monitoring of rodent colonies in animal facilities is essential to evaluate the health status of animals used in research studies. We examined the presence of selected microbial infections and parasitology contaminations in different types of animal facilities during 5-years screening at Tel Aviv University, which were monitored under *Federation of European Laboratory Animal Science Associations* (FELASA) recommendations. Results indicate that *Pasteurella spp.* was the most prominent bacteria found in Conventional and SPF units, followed by *Staphylococcus aureus, Klebsiella (pneumoniae, oxytoca*) and *Pseudomonas aeruginosa* which were found in lower indexes. Other isolated bacteria, not included in FELASA recommended panels, as *Proteus spp.*, *Enterobacter cloacae and Morganella morganii* exhibit minor frequency. Pinworms and mites, not found in SPF, showed prevalence ranged from 0.5 to 8% in conventional units. The SPF unit expressed a significant low incidence of isolated pathogens compared to conventional units, emphasizing the importance of a microbiological barrier created by the health monitoring standards in SPF. The present work highlights that in spite of vicinity of different animal facilities (SPF, conventional), their microbiological status could be sustained for long term when health monitoring, strict management, controlled personnel entrance, appropriate equipment (IVC cages, biological safety cabinets), restricted rodents transfers, the use of personal protective equipment and a prototype facility infrastructure were applied.

S2G1.2

A Mouse Is Not an Orange

K.P. Dhondt[1]
[1]*Charles River Laboratories - Research Models and Services, Saint-Germain-Nuelles, France*

Abstract
This 3-minute presentation will address two critical issues in laboratory animal research which may have a strong impact on breeding reduction, based on the concerning statistic of 8.3 million unused rodents and rabbits bred and not used in protocols in 2022 in Europe. The gender bias in experimental science will be discussed, emphasizing how unbalanced sex ratios in research can compromise reproducibility and drug efficacy. Exclusive data from Charles River's breeding sites will show progress in achieving balanced sex ratios in guinea pigs and rats, though challenges remain with certain mouse strains. Second, the presentation addresses the unnecessary euthanasia of animals with alopecia, particularly in C57BL6/J mice, where this condition is a normal characteristic as defined by the Jackson Laboratory. The speaker will then advocate for two key changes: maintaining natural sex ratios in research animals and accepting animals with normal alopecia, ultimately promoting more ethical and scientifically sound research practices.

S2G1.3

Development of a Climbing Test in Mice: A Novel Approach for Assessing Pain-like Behaviour

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Abstract
Introduction: Postoperative pain assessment in laboratory animals is crucial for securing their welfare, improving analgesic strategies, and developing novel compounds for pain treatment. This requires improved assessment techniques. This characterisation and validation study compared the von Frey test, a widely used assay for evaluating mechanical sensitivity, with a novel climbing test. The plantar incision model of postoperative pain was used to evaluate if a pain-related injury impairs climbing behaviour. For validation, the analgesic meloxicam was administered to revert surgery-induced behavioural changes.
Materials and Methods: Ninety-six C57BL/6JRj mice (48 males, 48 females) were used. Climbing and von Frey tests were conducted before and after surgery. Climbing behaviour was quantified using EthoVision®. In the validation experiment, meloxicam (10 mg/kg) was administered subcutaneously every 12 hours.
Results: The von Frey test demonstrated significant surgery-induced mechanical allodynia in both sexes, with no significant effect of meloxicam. The climbing test revealed reductions in distance travelled and the frequency of climbing in the top of the cylinder post-injury. Meloxicam-treated males significantly improved their climbing behaviour, while the effect in females was not significant.
Conclusion: We successfully demonstrated the climbing test as a viable tool for holistic and objective assessment of pain-related behaviours. Interestingly, it detected a significant effect of analgesia in males' climbing behaviour that was not found in the von Frey test. The reduced efficacy of meloxicam in females highlights potential gaps in sex-specific drug development and preclinical research. Further studies on analgesic sex differences and climbing behaviour of diverse pain models are warranted.

S2G1.4

Text-based Algorithms for Knowledge Management and Support of the Licensing Process of Animal Experiments

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[3]ETH Zurich, Center for Law & Economics, Zurich, Switzerland

Abstract
Animal experimentation and its licensing process are under pressure. In this field, a mismatch between available resources and the efficiency of knowledge management compromises fact-based decision making. This work evaluates the possibilities and challenges of alleviating the resource-knowledge management mismatch through a language model-based decision support system for the evaluation of applications for animal experiments.

Caveats of human decision making, i.e., lack of transparency, subdoxastic factors, and post-hoc rationalization, can be improved by implementing automated decision support systems. These systems may, however, induce changes in discretionary power and ambiguate liability. Requirements for the implementation of decision support systems in public administration include ethical and instrumental principles of human-centric and trustworthy data science. In addition, project success depends on organizational, technological maturity, and sociotechnical factors of technology implementation and requires a software-hardware-algorithm match.

In this work knowledge-based named-entity recognition was used to develop a proof-of-principle decision support system for authorities overseeing animal experimentation. The performance of the system was superior to that of NER by prompting a commercial large language model.

The following steps in developing and implementing a decision support system are delineated. It is essential to integrate stakeholders in system design. Developments in the fields of large language models and knowledge graphs may allow for improving the rule-based named-entity recognition established in this work. These methods may also allow improved efficiency of knowledge searches and knowledge integration in the licensing process of animal experiments.

S2G1.5

Refinement in Arthritis-spondyloarthritis Mouse Models

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[1]Institute of Fundamental Biomedical Research, Biomedical Sciences Research Centre 'Alexander Fleming', Vari, Greece

Abstract
Animal models of arthritis are commonly used to study the pathogenesis of the disease and establish translational platforms for the development of therapeutics and diagnostic biomarkers. At BSRC Alexander Fleming, we commonly utilize both spontaneous and induced animal models of arthritis-spondyloarthritis. In these models, the animals experience distress and pain, making it imperative to adhere to the principle of refinement to minimize suffering. While crucial for advancing our understanding of the diseases, it is essential to prioritize the ethical and humane treatment of the animals.

Here we explore current refinement strategies aiming to minimize pain and distress in mice employed in arthritis – spondyloarthritis research. These include incorporating environmental enrichment techniques, optimizing pain management strategies, and utilizing scoring systems to closely monitor animal welfare. Providing soft bedding and environmental enrichment, easily accessible food and water, and offering opportunities for natural behaviors such as social interaction is vital for alleviating pain and distress.

Suitable drug administration may delay or even prevent the development of clinically harmful phenotypes in genetically altered animals that spontaneously develop the disease. Refined mouse handling, using cupping and tunnels, should be preferred.

Finally, scoring systems, allowing identification of humane endpoints may prevent severe long-lasting suffering, especially regarding genetically altered animal models of progressive disease.

S2G1.6

The 3Rs in 3 Minutes: An Effective Mode of Communication?

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Abstract
The 3Rs of replacement, reduction, and refinement are vital principles to improving animal welfare within biomedical research. Within a large, global contract research organization, sharing information on improvements in the 3Rs from internal and external sources can be beneficial to overall culture of care. The Global Animal Welfare team has developed GAW Productions, with the goal to support a strong internal 3Rs program through multimodal communication to internal audiences. One type of production offered is a 3Minute3R with the aim to provide high level, quick communication to all employees from all levels, backgrounds, and educational experiences. The 3Minute3Rs aim to be 3–4 minutes in length and cover topics primarily related to animal biology and cognition that are tied back into the 3Rs principles. By covering these topics, the hope is to help strengthen employee respect and willingness to adopt the 3Rs for all species worked with at Charles River. 3Minute3R presentations are based off recently published peer-reviewed papers presenting empirical evidence and aim to be written for an 8th grade education level (70–60% Flesch reading ease). In 2024, 10 3Minute3Rs were released covering a variety of species including mice, zebrafish, dogs, cats, pigs, rats, and chickens. There was an average of 173 views per production, ranging from 119–266 views. User feedback will be presented on the benefits and areas for improvement for this mode of communication and its effects on promoting the 3Rs within a contract research organization.

S2G1.7

Reducing Severity with a Little Help from My Friends

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Abstract
Development and implementation of a standardized system for data management, related to animal welfare monitoring, is crucial for the ethical and scientific validity of animal research. At BSRC Alexander Fleming, online forms used by our team facilitate several key aspects of this process. It is noteworthy that these forms improve communication and collaboration among animal care staff, researchers, veterinarians and facility managers, significantly, ensuring timely and appropriate interventions. In addition, the generation of reports facilitates data analysis allowing continuous improvement." Daily care and observation" form for sick animals enables quick identification and treatment of animals in pain. A dedicated "flooded cage" form aims at immediate reporting of incidents, rapid identification of the cause, and preventative measures. "Animal welfare concern" form is used for reporting and investigating relative incidents. Monitoring equipment like autoclaves and anesthetic machines is used to identify and address issues that could compromise animal welfare and research data quality and validity. Digital tools and resources, as part of our environmental considerations strategy, are leveraged to reduce the need for physical materials, such as paper. With a little help from this simple tool, we aim for better animal welfare management, reducing severity.

S2G1.8

Fish First - Establishing New Rehoming Programs

H. Schöpper[1], J.C. Krüger[1] and B. Odermatt[1]
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Abstract
In order to enable laboratory animals to have a life worth living after experiments, institutions can rehome former lab animals to private owners according to EU Directive 2010/63 art. 17,19. Several requirements need to be met, as only healthy wildtype animals, that pose no harm to the public or animals health, nor environment may be rehomed. In addition, their expected life span needs to be in relation to the benefit of a new home and the potential stress of the rehoming process. In Germany a rehoming program including a habituation phase for the rehomed animal is required according to §10 TierSchVersV. Rehoming enables a sensible and accompanied release of former laboratory animals into private keeping. However, institutions with rehoming programs inevitably expose themselves to the public and sometimes fear negative effects of the press or others. In the area of tension between the desire to rehome animals that may benefit, and the potential to open up to criticism makes creating a rehoming program a balancing act. We are presenting a rehoming program for laboratory fish - a group of animals that is relatively easy to introduce into existing private aquariums, can be rehomed in larger groups at once and is not as emotional charged as other laboratory species kept as pets, such as mice or hamsters, for example. We state that institutions that want to gain initial experience with a rehoming program can find a favorable approach in the rehoming of laboratory fish species first.

S2G1.9

Introducing Sentinel-Free Health Monitoring - About the Importance of Modernizing Established Procedures

K. Weber-Wilk[1], T. Ganser[1], P. Follert[1] and S. Chourbaji[1]
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Abstract
At the Interfacultary Biomedical Research Unit (IBF) of the University of Heidelberg, we have recently reviewed and fully overhauled our long standing health monitoring regime.

The facility is mainly housing mice, in two large breeding units and one smaller experimental unit, with the animals being housed for the majority in open cages. We have decided to move away from traditional, dirty air sentinel-based health monitoring and towards fully sentinel-free and hybrid systems, primarily to reduce the significant number of animals used yearly for hygiene monitoring purposes, with the goal being to gradually move towards a completely sentinel-free system.

With this presentation, we aim to highlight the importance of challenging established procedures, implementing new research findings in the daily husbandry management and, ultimately, modernising processes.

Small changes can already make the difference and have a huge impact when addressing outdated practices.

S2G1.10

Implementation of the Replacement, Reduction, and Refinement Principles in Noninvasive Hypoxia Model in Rats

S. Trnski Levak[1], N. Jovanov-Milošević[1,2] and M. Judaš[1]
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[2]*Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia*

Abstract
Perinatal hypoxia and premature birth are significant contributors to neurological disorders and other physiological impairments in newborns, with effects that can extend into adulthood. To study these challenges, we developed a non-invasive rat model of moderate perinatal hypoxia, focusing on brain development disturbances and their long-term consequences using different research methods. Our research implemented a multimodal approach adhering to the 3R principles—Replacement, Reduction, and Refinement—allowing us to investigate mild to moderate brain deficits while minimizing the number of laboratory animals used and utilizing the same animals for various experimental processes. Given the lack of alternative models, we emphasized reducing animal numbers, reusing animals across various experimental procedures, and refining experimental methods during the implementation of the experiments. The same animals underwent

behavioral testing and were later used in non-return experiments for MRI and histological analyses. Our refinements included the use of non-invasive hypoxia induction and animal marking techniques, incorporating nest and maternal cage bedding during experiments, and ensuring minimal separation of pups from their mothers. We prioritized animal welfare through health monitoring, improved housing conditions, staff training, and the application of modern methods to minimize pain, suffering, and anxiety. These practices are integral to our commitment to ethical scientific research involving animals. The experiment designed in this way is an improvement of the previous animal models that are used to research perinatal brain injuries.

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S2G1.11

Refinement and Habituation of Golden Hamsters in Covid-19 Vaccine and Drug Research

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Abstract
Golden hamsters from European breeding stock are extremely friendly and easy to handle. We have used them intermittently as a model for SARS-CoV-2 infection. With the help of refinements, we were able to keep all procedures at a mild severity level and successfuly rehomed a number of them. Hamsters like to use tunnels to act out their natural behaviour and are very agile to turn around in confined spaces. In order to administer a controlled amount of the peptide drug with the nebuliser device, a tight animal holder tube on conscious animal had to be used. Two weeks of habituation were carried out to minimise the stress of being confined for 1–2 minutes per delivery session. Females were more responsive to food treats while males showed a pronounced olfactory exploration. Struggle to escape during actual procedure was only observed in few individuals in the last daily session and was not sex-specific.

S3A1.1

The Impact of Current Legislative Practices on Non-human Primate Research in Germany and Europe

K. Krug[1,2]
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Abstract
The European Union's Directive 2010/63/EU sets forth guidelines for the protection of animals used in scientific research, including non-human primates (NHPs). In Germany, the EU Directive has been transposed into federal laws, the Tierschutzgesetz (TierSchG, updated 2022) and the Tierversuchsverordnung (TierSchVersV, updated 2021). Additionally, the protection of animals is stated as a goal in the German constitution (Grundgesetz Art. 20a).

Individual German states ("Länder") are responsible for implementation of this legislation and administration of animal experimental permissions. This leads to challenges for animal research in Germany owing to the lack of an agreed interpretation of the legislation, which currently causes substantial delays in approval of protocols. Clearer guidelines on and definitions of severity classifications and, at least in some fields, community agreement on recommended protocols, such as Prescott et al. 2010 [1], could be helpful to the research community, support legislative practices and foster openness.

More generally for NHP research, we need to discuss openly how the current legislative practice across the EU might generate genetic bottlenecks in NHP breeding in Europe as well as potentially affect scientific and medical progress in specific fields. The lack of a programme for transgenic NHPs in neuroscientific and neuropsychiatric research in Europe could hinder medical advances. I will argue that there is also an ethical cost to delaying or preventing some animal research and we need to openly balance harms and benefits.

S3A1.2

From Policies to Public Opinion: Public Engagement and the Future of Non-Human Primate Research

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Abstract
The use of non-human primates (NHPs) in biomedical research is governed by stringent EU legislative principles (Directive 2010/63/EU), transposed in an even more restrictive manner by Italy (Decree 26/2014). This is largely due to the political influence and lobbying power of Italian animal rights groups, among the most organized in Europe. These groups secured a prohibition on NHP breeding, despite the absence of a ban on their use, necessitating imports and creating challenges in sourcing, delays in research, and a negative impacts on animal welfare. Although NHPs account for less than 0.01% of animals used annually in Italy—and 99% of them are employed to comply with EU and international regulatory requirements (particularly toxicity and safety testing)—the 0.0001% used in basic or translational neuroscience research is often emblematic of ethical debates surrounding animal experimentation, frequently labeled as "vivisection."

This contribution will examine the interaction between policy, the 3Rs (Replacement, Reduction, and Refinement), and public engagement regarding the (potential) future of NHP research. I will argue that combining Refinement of experimental procedures1 and transparency in communicating scientific results to the public, alongside the achieved scientific outcomes and their impact on advancement of knowledge and benefits on human health, can significantly shift public perception.

The adoption of new technologies and refined research strategies can also enhance the quality of scientific results while making them more easily communicable to the public, thereby

making this small yet indispensable component of biomedical research2 more sustainable and ethically acceptable.

S3A1.3

Coordination of Research Laboratories in Response to Current NHPs Legislative Practices

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Abstract
For many decades, non-human primates (NHPs) have been a key animal model in French biomedical research, from neuroscience to immunology, virology, or metabolic and respiratory physiology. The introduction of European Directive 2010/63/E and its transposition into French law have resulted in gradual changes in scientific practices involving NHPs and made the way in which this research is carried out and communicated transparently to the general public even more sensitive.

This presentation will illustrate how over the last ten years the creation of Biosimia, a France network for nonhuman primate biomedical research, has enabled the scientific community to coordinate its efforts in order to optimize their responses to regulatory, ethical and societal expectations. The Biosimia network has facilitated interaction between researchers, technicians and engineers in their ongoing quest to improve veterinary practices and animal welfare in laboratories, and to support their desire to communicate the challenges and objectives of their work. This network has not only reinforced the links between the various players in this field of research, but has also established itself as the point of reference for academic and research bodies, political decision-makers and the French centre for the promotion of the 3RS. It has certainly helped to strengthen France's position in NHP research in Europe, and we will be discussing the strategic relevance of extending this initiative at the European level.

S3A2.2

Assessment of Benefit from Ethics Committee's Perspective

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Abstract
In line with the Directive 2010/63/UE Directive, « it is essential to ensure that each use of an animal is carefully evaluated as to the scientific or educational validity, usefulness and relevance of the expected result of that use.» The Directive does not describe how to conduct an **Harm Benefit Analysis (HBA)** and how to make sure that benefits will truly outweigh the harm.

Scientists must avoid implementing unnecessary experiments (at best through systematic reviews) and use non animal based procedures whenever possible. **They are trained to design project using animals following the 3 R principles but also a critical evaluation of the need for animal studies first**. They must justify the choice of the model for the purpose of the project, describe the likelihood of achieving their goal and the primary benefits, the detailed techniques involved, their impact on the animal welfare and the means to reduce them.

The framework of the european non technical **summaries** has allowed to harmonize the contents of the projects and collect the informations that will facilitate the HBA. Current professional guidelines for HBA involve mainly a **qualitative approach**. Retrospective assessment of previous studies, pilot studies, regular reporting of postapproval monitoring as well as external expertise can help better evaluate the impact of the procedures on animal welfare and the potential to reach the goal of the project.

We will share experiences of HBA, the difficulties to set up a systematic approach to perform an objective assessment and to handle unfavorable analysis.

S3A2.3

Assessment of Benefit from Scientist's Perspective

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Abstract
Submitting animal research proposals to ethical committees is a complex and institution-specific process that demands thoughtful preparation and adaptability. Drawing from our experience submitting proposals to three distinct institutes in Athens, Greece, this talk will explore the challenges, lessons learned, and strategies for navigating these processes effectively. Each institute had unique expectations, requiring tailored approaches to proposal preparation. Understanding the audience—your ethical committee—is crucial. This involves collaborating with fellow researchers to gain insights into local practices, as well as ensuring you fully understand and can articulate your project's background and objectives. A robust harm-benefit analysis is central to the process. Beyond procedural harms, it is essential to consider all potential sources of harm throughout the animals' lifetime, such as housing, transport, and humane killing. Similarly, predicting the benefits of exploratory research requires thorough background research to ensure proposals are well-informed and scientifically justified. Timelines for submissions often conflict with funding agency deadlines, and while paperwork is generally consistent, the transparency of evaluation criteria varies across committees. Despite the administrative burden and differing practices, properly conducted harm-benefit assessments based on individual judgment and ethical committee reviews are important. When approached thoughtfully, they not only ensure compliance but also enhance the scientific rigor, ethical foundation, and reproducibility of research.

S3A2.4

Proposed Benefit Assessment Matrix

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Abstract
Evaluating harm:benefit tradeoffs in animal research is challenging. Although harms are often quantifiable (such as number of animals, cumulative severity and anticipated adverse effects), 'benefits' are more subjective and may not be of direct practical application; there may also be considerable differences between short-term and long-term harm:benefit tradeoffs. An international Harm Benefit Assessment (HBA) Working Group was convened in January 2023 to develop a Benefit Assessment Matrix as a practical tool for specific evaluation of benefit. The BAM tool emphasizes benefit associated with research process rather than criteria more appropriately assessed by ethics committees (such as animal-related care and study procedures). For a given research study, BAM computes a Qualified Benefit score based on Proposed Benefit (research gap addressed, anticipated scientific impact, lack of nonanimal alternatives) weighted by Modifying Factors (design validity; technical capability). High scores suggest the proposed research has a good probability of producing scientific benefit in terms of meaningful and reliable information, whilst minimizing waste of animals in nonproductive research. The BAM tool provides a streamlined and easily understood set of criteria for researchers, reviewers, and outside evaluators to provide appropriate and consistent evaluation of research quality.

S3B1.1

Comparative CNS Anatomy and Physiology

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Abstract
The translational validity of the animal models used in neuroscience and the subsequent harm benefit assessment are based on the knowledge of the anatomy and physiology of the laboratory rodents: the lissencephalic appearance of their brains is just one difference while structures including the olfactory bulbs (and rhinencephalon), the prefrontal cortex, hippocampus, basal ganglia and cerebellum are displaying differences that are crucial to determine the appropriate model, perform the complicated surgical techniques and/or assess the diverse behavioral tests used in neuroscience. Similarities and differences in the position of the tracts into the spinal cord are also critical for designing experiments, interpreting results, and ensuring ethical practices. In this presentation the fundamental differences of the most important CNS structures will be presented seen from an evolutionary perspective. Comparison on the relative brain size, brain mass, neuronal density and the cellular scaling rules will also be explained.

S3B1.2

Modelling CNS Diseases: In-vivo/ Ex-vivo/In-vitro Approaches and Their Translational Relevance to Human Neuroscience

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Abstract
Animal, and even human, models have been used for ages to disentangle the processes in the nervous system, as this is one of the more complex organs particularly in mammals. As science got more diversified, in vitro and ex vivo approaches were developed to tackled questions that do not need the live organ to be solved. Nevertheless, the complexity of the brain adds a barrier to this kind of approaches. In recent years, technologies like optogenetics and fiber photometry allowed to study the brain while animals perform different behaviors freely. Sophisticated approaches such as organoids representing minibrains have been conceived to overcome the use of animals. Still, some limitations reveals that we are far from debarrasing from living subjects in neuroscience. For example, the discovery of adult neurogenesis is considered one of the most important breakthroughs in neuroscience. Thus, studying the continuous generation of newborn neurons in the brain became an attractive target for the neuroscience community, but limitations to investigate the presence of new cells in the living brain remains a major drawbacks. Apart from the intrinsic factors modifying physiological neurogenesis, there are external regulators like stress, enrichment, or pathologies like epilepsia almost impossible to evaluate outside of the mammal brain. We will discuss such challenges to realistically progress towards a full «animal-free neuroscience».

S3B1.3

A Defined, Low Fibre Diet Makes EAE More Uniform and Allows Control over Severity

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Abstract
Myelin oligodendrocyte glycoprotein 35–55 (MOG35–55)-peptide induced experimental autoimmune encephalomyelitis (EAE) is a model for inflammation of the brain and spinal cord. However, its severity and incidence vary within and between laboratories. Severe scores can lead to premature termination and are both unnecessary for readouts and detrimental to animal welfare. Ideally, the model would have high incidence, moderate severity, and low interindividual variability to fulfill the "Refine" aspect of

the 3R concept. Nevertheless, most efforts to increase incidence also increase the severity. When the effects of potential therapies are tested, low to moderate severity is sufficient to detect useful drug effects as long as variation is low. Low variation can also reduce group sizes, which supports the "Reduce" aspect of the 3Rs. We set out to reduce variation and control severity by optimising mouse age, dietary fiber, antigen emulsion, and the dose of MOG and pertussis toxin (PTX). Our data suggest that using a defined low fibre diet makes responses more uniform with increased incidence without necessarily increased severity. This, in turn, allows the use of lower amounts of MOG or PTX such that predictable levels of severity can be obtained. These data suggest that adopting a specified diet will help laboratories run more comparable studies and reduce the number of animals required. More generally, these data suggest that responsiveness to auto-immune stimuli can be enhanced with diets that resemble modern human diets which may, in turn, have implications for translatability of drug response data.

S3B1.4

Innovations on Neurobehaviour (1) and Training and Competence in Interventional CNS Modelling (2)

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Abstract

Innovations on Neurobehaviour in CNS Modelling *JL Tremoleda*

Behavioral assessments play a crucial role in CNS modelling. A wide array of behavioral tests are employed in rodents to evaluate neurological traits such as locomotor activity, depression-like behavior, and cognition. However, these tests typically rely on the animal's response to specific tasks, often conducted in isolation and in unfamiliar environments. As a result, they can significantly affect the animal's emotional state, which in turn may influence the cumulative severity of the study. Home Cage Automatic Tracking systems enable the analysis of animals in their home cages, offering a more naturalistic, less intrusive approach to behavior assessment. We will discuss the challenges involved in evaluating the cumulative impact of repeated behavioral tests and explore paradigms that incorporate automated, naturalistic approaches to behavioral analysis.

Training and Competence in Interventional CNS Modelling *V Galligioni*

Interventional CNS work often implies complex surgeries and the use of ad hoc equipment/material (electrodes, in house-made implants, rings). These invasive procedures need surgical skills that are often not covered during normal LAS trainings. We will highlight the importance of involvement of technical staff and collaboration with other departments (AWOs, mechatronics for design of ad hoc tools/implants) to refine procedures, improve animal welfare and decrease the number of animals used for training.

All these interventions are commonly used in CNS research and we will reflect on their impact on the predictive validity of the models and importantly, on how its cumulative use is assessed during the harm-benefit analysis.

S3B2.1

INFRAFRONTIER - The European Research Infrastructure for Modelling Human Diseases

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Abstract

The modelling of human diseases is an important facet of modern-day biomedical research that contributes to better understanding of complex molecular pathophysiologies and identification of new therapeutic targets. In in vivo disease modelling, mice are popular model organisms due to their high genetic similarity to humans, occurrence of shared physiological pathways and biological processes and abundance of standardised gene-editing tools for genetic modifications. Their use has led to significant developments in the fields of cancer research, neurodegenerative diseases and infectious diseases and they have also contributed to several Nobel prize awarded studies.

INFRAFRONTIER is the European Research Infrastructure for modelling human diseases with a special focus on mouse models. Initially dealing with the generation, phenotyping, archiving and distribution of rodent models (mice and rats), it is currently evolving to include next generation of precision mouse models, preclinical disease-oriented pipelines and cutting-edge complex in vitro models (CIVMs) into its portfolio with the help of newly acquired Horizon Europe funding. With this support from the EC, INFRAFRONTIER will promote the application of the 3R Principle in the development of next generation disease modelling technologies and services. These activities include the establishment of a pilot mouse biobank, upgrading its data curation and enrichment activities, designing and applying machine learning-assisted data analysis tools, and creating a comprehensive in vitro resource for preclinical research. With these developments, INFRAFRONTIER aims to bring the 3Rs into the new era of disease modelling.

S3B2.2

Linking State-of-the-Art Cryopreservation to Animal Welfare

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Abstract

The European Mouse Mutant Archive (EMMA), the mouse repository within INFRAFRONTIER, is now the third largest non-profit repository worldwide. By making cryopreserved mouse lines publicly available, it contributes primarily to the point reduction within the 3Rs.

Moreover, the focus of all 11 EMMA nodes involved has been on the refinement and standardization of the techniques used. The goal was to use the best and most robust methods, from importing the animals required for cryopreservation to quality control of the cryopreserved materials, to reproducibly achieve an optimal result

with as few animals as possible, thus contributing to reduction. Both the so-called CARD and JAX methods for sperm cryopreservation have proven to be excellently suited for this purpose. The collaboration of the EMMA nodes enabled a unique exchange of experiences and the testing of new methods to prove, for example, the safety of international sperm shipping on dry ice or the shipping of refrigerated epididymides and embryos instead of live mice. If the reproductive characteristics of a mouse strain are known, blastocyst genotyping can be used for quality control, thus avoiding the production of unnecessary offspring through embryo transfer. All methods have been published and made available as Standard Operating Procedure (SOP) to the scientific community via www.infrafrontier.eu. Efforts are now being made to develop and apply binding quality control principles across all nodes. All efforts thus contribute to the cryopreservation and worldwide distribution of mouse models with as few mice as possible and thus to the fulfillment of the 3Rs.

S3B2.3

How can we apply Reduction and Refinement in Systemic Analysis of Mouse Phenotyping?

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Abstract
INFRAFRONTIER is the European Research Infrastructure for modelling human diseases, dedicated to generation, phenotyping, archiving and distribution of rodent models. In particular, characterization of genetically modified rodent models for human diseases has become a key tool in basic and biomedical research to understand molecular mechanisms of human disorders and to develop therapeutic strategies.

The **Institut Clinique de la Souris (ICS)** has been created in 2002 and is a main node of PHENOMIN-CELPHEDIA, the French National Infrastructure in Biology and Health. ICS is a key founding member of INFRAFRONTIER, and a major player in the international phenogenomics effort, contributing to the International Mouse Phenotyping Consortium (IMPC) to decipher gene function of every gene in the mouse genome.

Systemic phenotyping pipelines have therefore been developed, covering multiple organs and physiological systems through a wide range of tests to identify the phenotypes. However, the complexity and variability inherent in phenotypic data require approaches such as Reduction and Refinement to optimize the protocols and improve quality and reliability of research outputs.

Here, we will illustrate how to integrate reduction and refinement strategies into systemic mouse phenotyping, by improving experimental design, using non-invasive technologies or automated systems, and optimizing housing conditions and animal handling.

By incorporating these approaches, more precise and meaningful phenotypic analysis can be achieved with fewer animals, while improving data quality and ethical standards. Ultimately, the successful application of reduction and refinement techniques in phenotyping studies will lead to more efficient and humane models for understanding genetic diseases and developing therapeutic strategies.

S3B2.4

Replacement of Mouse Models by Using Organoids and 3D-Bioprinting in Basic Gastrointestinal Research

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[3]*Helmholtz Zentrum München, Neuherberg, Germany*

Abstract
For decades, mice, and especially genetically modified mouse models, have been indispensable in biomedical research. In the field of gastrointestinal research, specific mouse models are also widely used for basic research as well as for drug development and testing. However, in recent years, several alternative methods have proven to be viable substitutes for mouse models. The advent of CRISPR-Cas9-technology has provided a highly reliable tool for the precise and stable genetic manipulation of cells, reducing the reliance on genetically modified mouse models. Additionally, organoids enable researchers to conduct studies using three-dimensional cell models, while 3D-bioprinting offers a cutting-edge-technique for arranging cells in an ink to replicate tissues. INFRAFRONTIER, the European research infrastructure for modelling human diseases, dedicated to generation, phenotyping, and archiving of mouse models, is leveraging these advancements through its new INFRAPLUS and PRIM-TECH3R projects. These initiatives aim to utilize INFRAFRONTIER's resources to develop alternative models that replace or reduce animal testing and make them accessible to scientists. As part of these efforts, one project focuses on developing a replacement method for basic research on pancreatic ductal adenocarcinoma (PDAC) and creating a reliable *in vitro* model for testing potential therapeutic substances against PDAC. In our laboratory, one PDAC mouse model is being replicated in vitro using 3D-bioprinting. In another project, alternative models are being designed for inflammatory diseases of the gastrointestinal tract. The goal is to develop substitutes for acute colitis and acute pancreatitis by generating organoids, thereby eliminating the need for animal testing in this area of research.

S3C1.1

Comparative Analysis of Different Minimal-Invasive Sampling Methods for Genotyping: Improvement Towards the 3R Principle

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Abstract
The correct genetic identification of rodents is essential to the efficiency and reproducibility of research. Moreover, accurate genotyping, traditionally reliant on invasive ear and tail biopsies, plays a critical role in reducing both the number of animals used and the associated time and costs. In regard to the 3R Principle, we continuously strive to optimize our procedures and enhance animal welfare within research.

In a pilot study, we compared different minimal-invasive with invasive sampling methods to see whether these methods offer a reliable alternative to traditional methods. Regardless of the sample type, the DNA is extracted and then amplified using conventional PCR followed by capillary gel electrophoresis or real-time PCR methods. Our findings demonstrate that purified DNA extracted from oral swabs, hair follicles, feces, and dried tears led to clear target signals comparable to those obtained from their corresponding biopsies.

Feces offer a promising alternative, as they are routinely collected for the health monitoring at Charles River, eliminating the need for additional training. Additionally, all minimal-invasive sampling methods, except for hair, exhibit a reduced risk of cross-contamination compared to biopsies.

With consideration to the 3R Principle and a growing interest in refining genotyping methods, we have optimized our different minimal-invasive sampling methods to match the efficiency of invasive methods. These sampling methods provide an alternative to traditional methods and can be used for large-scale routine genotyping, especially for animals with ear tags, tattoos, or if a second biopsy is not allowed.

S3C1.2

Shake It, Baby! Lessons from the Conflict between Construction Sites Next to Animal Facilities

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Abstract
We suffer the problem of several major construction sites next to our animal facility. Since this scenario is a problem at most research campuses, we want to share our experiences, which construction activities have shown to be especially detrimental for our mice, what impact acute and chronic stress caused by the construction activities has on the affected animals and which solutions there are to attenuate the most detrimental effects on the animal facility.

At the beginning of the construction campaign several test fields were set up next to our animal house, to measure the effect of different simulated construction activities. Additionally, by using a specialised measurement device for animal houses we can directly link particular construction activities to their effects on our animal facility. Recorded by this device were noise and vibration of the building directly on-site in the animal facility. As acute response to the construction activities our animals exhibit more fighting, spontaneous deaths, massive decline of breeding performance and barbering. Animals that have been subjected to the construction stress for a prolonged period can develop seizures upon triggering with a stressor.

Particularly vibrations caused by the construction activities have a massive impact on the mice, up to impending loss of complete mouselines. We could not identify any solutions to protect our animals against these vibrations. However, we could identify, which construction equipment and -techniques cause the most vibrations and should therefore not be employed close to the animal facility, and which techniques have proven to be relatively innocuous.

S3C1.3

Vivarium Rotation Program: Advancing Animal Welfare, Fostering Cross-Vivarium Collaboration, and Global Culture of Care

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[4]Merck, Rockville, United States
[5]Merck, Edinburgh, United Kingdom
[6]Merck, Rehovot, Israel

Abstract
Our Vivarium Rotation Program (VRP) is an innovative initiative designed to break silos and maximize exchange, enhance employee skills, promote collaboration, and advance animal welfare. Tailored to all roles involved in animal work - the program facilitates transparency and knowledge acquisition, strengthens adaptive capacity, and fosters a global Culture of Care.

The program includes six vivariums in five countries participating in a rotation, where nominees from each vivarium travel to another site every year. Participants immerse themselves in the host vivarium's practices, learning new approaches and experiencing different ways of working. This cross-site exposure encourages exchange of best practices, knowledge transfer, and adaptation of innovative solutions tailored to local regulatory and ethical requirements. Participants gain hands-on experience in all operational area and deepening their understanding of ASW principles. The participants become part of a sustainable expert network

within the VRP Community. After completing their journey, they report to this Community and the Vivarium Board on what they have gained and contributed. This approach makes the program's impact both measurable and visible.

Evaluation of the program demonstrated significant benefits, including enhanced knowledge acquisition, staff proficiency, adaptive capacity, and a stronger commitment to animal science and welfare. Participants also expressed a sense of belonging to a unique and collaborative community, driven by shared learning experiences and a unified mission to improve animal welfare practices.

By embedding cross-vivarium collaboration, we enhance transparency and drive innovation, ultimately improving efficiency, enabling better decision-making, and increasing organizational agility in all aspects of in vivo work.

S3C1.4

Evolution of Murine Microbiome from Vendors, Quarantine, Germ-free, Breeding and Experimental facilities over 24-Weeks

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Abstract

Recent evidence highlights the critical role of the faecal microbiome (FM) in human and animal health, as its impact on experimental reproducibility and translatability. Emerging studies suggest that differences in phenotype expression observed after mice transportation are linked to FM shifts. However, unlike standardized health reporting, FM analysis in laboratory mice remains frequently unexplored.

This study investigates FM variations in mice housed at Bern University Experimental Animal Center (EAC) facilities, where differences in husbandry practices, personnel and sanitary conditions, may influence gut microbial populations. Through shotgun metagenomic sequencing (TransnetYX©) and One Codex© software, the authors assessed the evolution of murine FM across six original baselines, with a total of 204 samples, tracking changes over a 24-week period. Mice were housed in four EAC facilities (SPF, SOPF, and germ-free) and either bred internally or imported from two commercial vendors. Results revealed 1) Significant origin-dependent selective colonization at baseline. 2) mice's guts were all well-colonized with exception of the germ-free colony. 3) microbiome source-fingerprint is facility specific and 4) FM remained stable over the 24-week time despite moving to another facility.

These results align with prior studies showing that mice from different vendors or facilities exhibit unique FM profiles, emphasizing the influence of host-related and environmental factors on microbiome composition. To improve reproducibility in murine research models, the authors advocate for mandatory profiling and publishing of microbiome fingerprints. This study highlights the necessity of incorporating FM analysis into laboratory protocols to enhance experimental reliability and encourage advancements in One Health.

S3C1.5

Low-cost AI Smart Cages to Digitize Existing Infrastructure

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Abstract

Home cage monitoring has the potential to revolutionize animal care and scientific research. However, three challenges have prevented large-scale use: high costs, difficulty of altering existing infrastructure and the need for simultaneous multi- animal tracking.

To address these challenges and enable wide-spread use of smart cages, we designed DOMEs - "smart lids" that fit above existing cage bottoms in existing racks. The smart lids record video and audio, which is processed through AI computer vision pipeline to identify and track group-housed animals. Thanks to low-cost hardware as well as software optimizations, this system is 30x cheaper than current dominant smart cages.

Using a new multi-animal tracking software, the smart lids give access to 18 health metrics, including activity, sleep, aggression, eating & drinking. The system also continuously tracks the condition of the cage, sending automated alerts in case of cage condition problems or health issues.

By solving the problems of cost, compatibility with existing infrastructure and multi-animal tracking, we aim to offer a solution that can be used to improve animal welfare and research widely, across institutions with different infrastructure and budget capacities.

S3C1.6

Enhancing Training and Reducing Animal Use: 3D-Printed Rodent Tail Models for i.v. Injection Training

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Abstract

Intravenous (i.v.) injections are widely used in mice for substance delivery, yet their success heavily depends on the operator's skill and experience. Due to the limited number of accessible veins in mice, achieving reliable results often requires extensive training

on several live animals. Novice personnel, in particular, require even more animals to familiarize themselves with tail and syringe handling before focusing on the injection process itself. Training on artificial mouse tail models offers an opportunity to better prepare trainees in terms of handling and efficiency while also reducing the number of animals needed for training. However, existing training models often lack anatomical accuracy and realistic tactile feedback. In response, a novel 3D-printed mouse tail model has been developed, designed to replicate the anatomy and tactile properties of a real tail more effectively. User feedback suggests this enhances i.v. injection training success and highlights a strong demand for additional artificial animal models to support technical skill development. Although 3D-printing provides a versatile platform for creating such tools, the process requires substantial prototyping, testing, and quality control to ensure usability and reproducibility. These challenges underscore the complexity of developing and distributing reliable training tools, despite the apparent simplicity of sharing and replicating 3D-printing designs.

S3C2.1

The 3Hs Initiative: Introduction to the Concept and Framework

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Abstract
By addressing the animal's relationship to both its environment and research staff we can reduce the negative impacts of cumulative stress across the entire lifetime of the animal. We have developed a framework that considers the lifetime experience of the laboratory animal and seeks to improve their welfare by targeting the 3Hs: Housing, Handling and Habituation. This talk will introduce the framework, including objective methods we have developed to quantify rodent affective state and the critical role positive reinforcement plays in habituation. The talk will also consider how the framework can be applied to species beyond rodents, including larger animals.

S3C2.2

Apply the Principles of the 3Hs for the Management of Laboratory Rodents

J. Bartlett[1]
[1]*University of Bristol, Bristol, United Kingdom*

Abstract
This talk will introduce some methods for implementing the 3Hs principles. If we can reduce or remove the stress caused by routine handling and during experimental procedures, then we can have a significant impact on the cumulative stress that the animal experiences over its lifetime. If an animal forms a positive association with human contact, then the cumulative impacts of routine care and procedures can benefit their welfare rather than add to their suffering. The benefits of habituation may also be felt by the animal care staff and researchers as positive human-animal interactions may be linked to decreased compassion fatigue in laboratory animal workers and increased satisfaction in their work. During experimental procedures the instinct a lot of the time is to restrain the animal as securely as possible as this is perceived to be better for the safety of the animal and the handler and the accuracy of the procedure, but physical restraint is highly aversive for animals and the negative impacts of restraint do not seem to decrease with repeated exposure although animals may exhibit passive coping strategies such as learned helplessness when overt signs of distress decline, but the affective experience does not. The stress caused to the animal will have an impact on the results of your study as well as the welfare of the animal handler. Instead, maybe we will give examples where we are using the least amount of restraint to achieve the outcome we need, or even removing the need for restraint altogether.

S3C2.3

Use of Behavioural Training and Habituation to Support Refined Methods in Large Animal Species

C. Juel Bundgaard[1]
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Abstract
The presentation will focus on the benefits of implementing a routine socialization program tailored to the specific research requirements of minipigs, pigs, rabbits and dogs thereby eliminating the need for physical restraint and use of force during procedures and reducing fear responses during handling. By tailoring the socialization program to the distinct research needs, the animals become acclimated to human interaction, facilitating stress-free handling. Furthermore, the presentation will give examples of housing and environmental enrichment of minipigs, pigs, rabbits, and dogs in a way where the animals needs are taken into consideration to give the animals a positive welfare. The importance of training animals for technical and husbandry procedures will be described and examples of specific training are provided. The advantages of such an approach include voluntary participation of the animals in procedures and minimal stress for both the animals and the staff responsible for their care. This presentation aligns with the 3Hs theme (housing, handling, and habituation), shedding light on the pivotal role of refined behavioural training and habituation methods in promoting the welfare of large animal species within research settings.

S3C3.1

Introduction to Clinical Score Sheets

M. Heimann[1]
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Abstract
Clinical Score Sheets are important tools designed to systematically assess and document the welfare of laboratory animals. Their use is highly recommended because they provide a structured, objective, and repeatable way to evaluate health and welfare parameters, thus ensuring consistency and accuracy in assessments. Without the use of Clinical Score Sheets, assessments can become subjective, inconsistent, and overlooked, potentially leading to compromised welfare, inappropriate interventions, and ethical concerns.

While there are many published clinical assessment sheets, their universal applicability is limited due to differences in procedural contexts, animal species, and specific care requirements. This highlights the importance of developing customised clinical evaluation sheets that are tailored to the unique needs of each project. Customisation ensures that the Clinical Score Sheets are practical, relevant and effective, enabling accurate monitoring and informed decision-making that meets the highest standards of welfare.

S3C3.2

Clinical Score Sheets: Friend or Foe

D. Bonaparte[1]
[1]*Royal Netherlands Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands*

Abstract
Clinical score sheets are indispensable tools for safeguarding the welfare of animals used in research, enabling systematic and objective monitoring of their health and well-being and proposing mitigation measures to reduce constraints. Well-designed score sheets allow the prompt detection and response to animal discomfort, thereby minimising suffering and aligning with the 3Rs principles.

However, the benefits of clinical score sheets may be compromised by their miss-use. Ambiguous or overly complex criteria can lead to inconsistent assessments and delayed interventions, while generic templates may fail to capture species- or procedure-specific welfare indicators. Poorly designed score sheets can also create unnecessary administrative workload or lead to subjective evaluations, undermining their intended purpose. Such issues may result in unrecognized distress, compromised data integrity, and non-compliance with ethical guidelines.

This session will focus on the problems deriving from the use of inadequate clinical score sheets, either poorly designed or simply not adapted to the specific context. For a better engagement, this session will draw from common, real-life examples of miss-using Clinical Score Sheets, which the audience will be invited to review. By emphasising what can go wrong, this session aims to be a wake-up call for the audience to be more critical in designing, reviewing and using Clinical Score Sheets.

S3C3.3

The Score Sheet - A Powerful Tool if Properly Designed

P. Bugnon[1] and M. Heimann[2]
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[2]*Swiss Institute of Technology ETHZ, Zürich, Switzerland*

Abstract
Score sheets are essential tools for evaluating the impact of experimental procedures on animal welfare. When designed effectively, they ensure that researchers can accurately assess the well-being of animals, define early intervention points, and document experimental procedures. However, the effectiveness of score sheets depends on incorporating key principles to fulfill their intended purpose. Poorly designed score sheets not only waste valuable research time but also fail to provide an accurate evaluation of the constraints imposed on animals. This lack of accuracy can lead to significant negative consequences for animal welfare, undermining both ethical standards and scientific validity.

Well-constructed score sheets not only facilitate a thorough assessment of animal welfare but also enable early detection of adverse conditions, reducing the overall severity of experiments. By defining clear intervention points, they help mitigate negative impacts, ensuring better outcomes for the animals involved. Furthermore, these tools are invaluable for maintaining detailed records of experimental procedures and outcomes, which contributes to transparency and reproducibility in research.

This presentation will explore the critical elements required for developing effective score sheets, emphasizing their role in advancing both animal welfare and scientific rigor. Participants will gain insight into best practices for score sheet design and their practical applications. Additionally, attendees are encouraged to join the workshop *"Self-made Score sheet or not? That's the question."* This in-depth session provides a step-by-step guide to designing customized score sheets tailored to specific experimental needs.

S3C3.4

CSS: Can We Wear *Prêt-a-Porter and* Still Look Good? Example from a Multicentre Institution

D. Bonaparte[1]
[1]*Royal Netherlands Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands*

Abstract
Generic Clinical Score Sheets may miss important welfare indicators, leading to inaccurate assessments and delayed interventions. In the previous sessions, the need to use customised Clinical Score Sheets that are fully aligned with the specific needs of the target animals, the procedures and the research context was largely discussed. By specifically designing score sheets, veterinarians, researchers and welfare staff can ensure that the welfare of animals is closely monitored and accounted for.

But must every single Clinical Score Sheet be tailor-made? Or is there room for some "prêt-a-porter"?

This session will share the example of a multicentre institution where several new study protocols are submitted for review and approval by the Animal Welfare Body every week. With only one veterinarian and no prior culture of using properly designed Clinical Score Sheets, a strategy was implemented to optimise resources while ensuring the use of well-defined welfare monitoring and scoring criteria, as well as moments to intervene and actions to take. One year later, the use of tailored Clinical Scoring Sheets is now generalised, and the researchers are much more critical when defining welfare criteria. This strategy reduced the workload on the Animal Welfare Body, resulted in a better engagement of the research teams and welfare care staff and ultimately led to better animal welfare and better science.

S3C4.1

Systematic Review of Guidelines for Good Surgical Practice in Experimental Rodent Surgery

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[2]*University of Zurich, Zurich, Switzerland*
[3]*AO Research Institute, Davos, Switzerland*

Abstract
Surgery is an integral part of many animal studies. Good surgical practice (GSP), including aseptic and minimally invasive techniques and optimal peri-and post-operative care, is a prerequisite to achieve surgical success and optimize the animal's post-operative recovery. Moreover, using GSP can reduce variability, improve reproducibility, and increase the external validity of an experiment. However, there seems to be a lack of implementation of GSP especially for rodents. Since guidelines help to adhere to certain procedures, we aimed at identifying, critically evaluating and comparing available guidelines describing surgical practice for experimental rodent surgery.

In accordance with our pre-registered protocol, we comprehensively searched PubMed, Embase and Web of Science for guidelines on rodent surgery. 1585 unique results were retrieved, of which 29 publications met the inclusion criteria. In addition, unpublished guidelines were identified by searching in Google, laboratory animal science books and a survey among researchers. A descriptive synthesis of the continent of origin, year of publication, prevalence of unique individual recommendations, and a qualitative comparison of the recommendations was done.

Many key points have been recommended by most but not all guidelines. However, the basics of aseptic surgery should be mentioned in every rodent surgery guideline, while recommendations deviating from standard surgical practice should be avoided. Guidelines should address all relevant key points in a clear and well-structured manner to provide concise guidance and specifically consider the surgeons' background. Efforts should be made to agree on the best standards for rodent surgeries and harmonize guidelines accordingly.

S3C4.2

The OBSERVE Guidelines: Oncology Best-practices: Signs, Endpoints and Refinements for in Vivo experiments

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[6]*Genmab, Utrecht, Netherlands*
[7]*Janssen Pharmaceutica, Beerse, Belgium*
[8]*University of Veterinary Medicine, Vienna, Austria*
[9]*PSL Research Univeristy, Paris, France*

Abstract
Several publications provide guidelines on the preparation (PREPARE) and reporting (ARRIVE) of animal experiments. Nevertheless, a clear and standardized approach during in vivo cancer studies is still lacking, with the content of most publications proving too general. Moreover, as the EU and its Member States has mainly focused on reduction and replacement, here we closely consider the 3rd R, refinement, which is insufficiently considered. With OBSERVE, we offer robust and practical recommendations on refinement approaches for both scientists and animal care staff involved in cancer research, an initiative supported by EurOPDX and INFRAFRONTIER. The foundation of OBSERVE is built on tumour-specific clinical signs ensuring the guidelines have utility across a wide variety of animal models regardless of tumour origin (genetically engineered models (GEMs), patient derived xenograft (PDX), etc.). In a stepwise manner, we comprehensively address refinement approaches and monitoring during various aspects of murine cancer studies. First, we elaborate on appropriate preparation of tumour cells/-fragments, and refinement of tumour implantation methods. Next, we describe clinical signs associated with a panel of tumour categories. The primary aim of OBSERVE is refinement during tumour development: monitoring of tumour growth and appropriate follow-up of animals during tumour development. The latter is achieved through a comprehensive set of monitoring sheets tailored to varying clinical signs including specific humane endpoints. Finally, an overview of severity assessment in relation to clinical signs, implantation method and tumour characteristics is presented. Overall, we aim to provide oncology researchers clear and robust guidance on in vivo cancer model refinements.

S3C4.3

Repetitive Isoflurane Anaesthesia Alters the Behaviour and Brain Cellular and Vascular Integrity in Mice

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Abstract
Isoflurane is widely used to induce anaesthesia in laboratory mice, with many experimental procedures requiring repetitive short exposures during imaging and other interventions. Although isoflurane is generally considered a safe agent, human studies suggest that repeated exposure to volatile anaesthetics can lead to long-term cognitive effects. This study investigates the impact of repeated isoflurane exposure on CD1 adult mice (n = 12, balanced for sex).

Mice were exposed to isoflurane six times for 10 minutes each, with intervals of 2–3 days between exposures. Using the Actual Analytics Home Cage system, we monitored individual activity within group-housed settings, body temperature, and a battery of social and maintenance behaviours over an 18-hour period following each exposure period. At 48 hours after the sixth exposure, brain samples were analysed for evidence of microglial activation to assess acute neuroinflammatory responses and vascular integrity.

Following repeated isoflurane exposure, mice exhibited regular circadian activity and body temperature fluctuations. But both sexes showed a significant decrease in burrowing behaviour. Brain analyses revealed dysregulation of microglial resting states, characterised by a downregulation in pro-inflammatory marker expression. Additionally, vascular studies indicated significant alterations in vessel wall proteins, with further investigations into the impact on cerebral blood flow currently underway.

These findings highlight the short-term effects of repeated isoflurane exposure in mice, emphasizing the importance of closely monitoring recovery periods between exposures to support balanced tissue homeostasis and recovery. This may be particularly relevant when considering the cumulative effects on the CNS and behavioural outcomes

S3C4.5

Development of a Neonatal Piglet Model for Brain Injury Assessment in Cardiac Surgery

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[5]*Department of Pediatric Cardiology, Hospital Sant Joan de Déu, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain*

Abstract
One severe complication of neonatal cardiac surgery is brain damage. To develop devices for these surgeries, a reliable piglet model of cardiopulmonary bypass (CPB) with circulatory and cardiac arrest (CCA) is essential.

For this study, 30 neonatal piglets (4–6 days old, 2.5–3.5 kg) were used. Baseline brain 3T-MRI scans were obtained, and histological and biochemical analyses were performed to assess brain damage through tissue, blood, and urine biomarkers. The right femoral artery and vein were cannulated for pressure monitoring and vascular access. Cannulas were placed in the right internal carotid artery and left external jugular vein and connected to the CPB circuit. Potassium chloride induced cardiac arrest, confirmed by echocardiography. CPB flow was stopped to induce circulatory arrest, with body temperature either maintained at normothermia or reduced to 18–20°C (hypothermia) for 30–60 minutes. Afterward, body temperature was restored to 38°C, circulatory flow and ventilation were reestablished, and resuscitation began.

After 120 minutes, piglets were weaned off CPB, decannulated, and a follow-up 3T-MRI was performed. The mean time from sedation to CPB cannulation was 3.2 hours, with successful CPB and cardiac arrest in 92% of experiments.

In conclusion, this model is reliable, generalizable, and suitable for research. The use of state-of-the-art facilities with advanced technologies, significantly enhances the precision of the study, reduces the number of animals needed, and improves animal welfare. Moreover, experienced staff are crucial for optimizing the model's success and minimizing any potential harm to the animals, thus ensuring ethical standards are met while advancing scientific research.

S3D1.1

15 Years of AWB in Europe

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Abstract
Over the past 15 years, following the adoption of Directive-2010/63/EU, the Animal Welfare Bodies (AWB) in Europe have significantly enhanced the ethical oversight of using animals for scientific purposes. The principal tasks of the AWB are providing advice on matters relating to animal-welfare and fostering a culture of care. This presentation will evaluate the progress made by AWBs across European institutions, including challenges encountered and future directions.

AWBs have played a pivotal role in providing ethical advice, reviewing processes relating to welfare, and promoting the 3Rs. Recommended multidisciplinary composition should ensure diverse expertise, balancing scientific objectives with welfare considerations.

Significant advancements in training and implementing refinement techniques have been achieved. The emergence of new technologies, such as organ-on-chip systems and AI, presents opportunities to further minimize animal use. Yet, challenges persist, including the ability to keep up with developments in alternative methodologies, resource constraints, administrative burdens, and a lack of collaboration among AWBs.

The Directive was intended to harmonize standards across member states, improving transparency and public accountability. However, implementation has been uneven, with variations in resources, enforcement, and interpretation of the Directive across countries, including around AWBs.

Looking forward, it will be essential to strengthen the communication and networking, while ensuring the resources needed for effective operations. This 15-year milestone underscores the need for sustained commitment to the AWB, fostering innovation and ethical integrity in scientific research. The empowered, and connected, AWB should be a cornerstone in Europe's efforts to ensure responsible and humane treatment of animals in science.

S3D1.2

How the National AWB Networks Have Built Together a European Network

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[2]*RSPCA, Horsham, United Kingdom*
[3]*–, Maaloev, Denmark*

Abstract
At the 2022 Federation of European Laboratory Animal Science Associations (FELASA) congress, a plenary session discussed how national Animal Welfare Body (AWB) networks can improve promotion of a good Culture of Care by sharing initiatives and best practices. Several countries have set up AWB networks, enabling AWBs to share good practice with respect to establishing effective processes and fulfilling all of their tasks. This has helped many AWBs to improve animal welfare, the implementation of the 3Rs, the quality of the science and staff well-being. Despite these benefits, organized national AWB networks are not yet common practice. A group has been established with the aim of encouraging and developing more national AWB networks. To full fill this objective a core group, has started an European Network of National Networks for AWBs' – ENAWB- to help ensure good practice for achieving the following:

- All AWBs successfully meeting the requirements of Directive 2010/63/EU.
- AWBs supporting one another, learning from one another and sharing experiences on how AWB tasks are successfully delivered.
- Sharing of practices and ideas on operational matters, regarding national AWB networks.
- Discussion of AWB operational issues and challenges, and how these can be addressed.
- Sharing of training materials for AWBs.
- Enabling AWBs to address the 3Rs more effectively.

ENAWB that now has the representation of 10 countries, will present the network's activities, proposals for its further development and plans for future joint initiatives.

S3D1.3

RedeORBEA -The Portuguese Animal Welfare Bodies Network. A Sum Greater Than its Parts

N.H. Franco[1] and A.I. Santos[2]
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[2]*NOVA Medical School, Lisbon, Portugal*

Abstract
With 2010/63/EU, European establishments were required to have Animal Welfare Bodies. However, there was no previous experience in Portugal of bodies with such competences and attributions, which raised challenges regarding defining their mission, finding their place, and establishing their authority within institutions, while navigating new regulatory requirements.

Faced at the time with little to no guidance from the authorities in this regard, laboratory animal scientists and professionals from across the country gathered twice in 2014 to discuss how to start AWBs in their establishments, and the idea of harmonizing procedures and working together as a network was born. Following the first National Symposium for Portuguese AWB (the ORBEA) in 2016, the RedeORBEA was constituted, with an informal and decentralized model of organization, with no membership nor fees, and with two co-opted coordinators. RedeORBEA holds regular surveys and censuses, disseminates information and training opportunities, and organizes courses and workshops, as well as annual symposia (with the 10th edition being prepared in 2025), supported by SPCAL.

RedeORBEA provides a safe and welcoming forum within which AWB members can freely ask questions and exchange experiences with peers with the same responsibilities and facing the same challenges, and act in unison when interacting with other stakeholders, including authorities.

RedeORBEA has allowed raising and harmonising standards of competence and functionality in the planning, following, and evaluating projects and procedures within institutions, raising and harmonizing principles and practices for the benefit of both animals and the humans caring for them, promoting a true culture of care.

S3D1.4

The French Guide for the Animal Welfare Bodies

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[6]*CEA, Grenoble, France*

Abstract
The French Network of Animal Welfare Bodies (RN-SBEA) was established in 2019, following the identification of the need for a national network for animal welfare bodies (AWB). The network aims to facilitate the sharing of experiences, resources, and best practices among participants.

After more than five years, the RN-SBEA now includes over 400 AWBs. It has held five annual meetings and distributed more than 30 newsletters. From the beginning, the network set various objectives, with the primary mission of providing services to AWBs through the development of activities that help AWBs and their members improve their performance.

The main project of the RN-SBEA was to write and publish a "Guide" to help AWBs be effective, particularly regarding their regulatory missions. After 1.5 years of collaborative work, this Guide was finally published in June 2024.

This talk will present the process of writing the Guide and its content, hoping to inspire other AWB networks to follow this path. More than 50 persons have contributed to the writing, including AWB members and veterinarian inspectors. These contributors shared their knowledge and expertise coming from their daily activities.

The network receives the support of the 3 associations which contributed to its creation: AFSTAL, GIRCOR and OPAL.

S3D2.1

Pioneering Transparency in Animal Research: Promoting Openness, Ethical Accountability and a Culture of Care

K. Leech[1]
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Abstract
Responding to a need for open dialogue about the societal benefits of biomedical studies using animals, EARA has collaborated with institutions across Europe to develop national Transparency Agreements (TAs). These agreements have become instrumental in addressing public concerns and misinformation while promoting ethical accountability. Since the first TA was launched in the UK in 2014 by Understanding Animal Research, EARA has supported their global expansion. Now spanning eight European countries and two outside Europe, and reaching over 600 institutional signatories, the TAs highlight a collective commitment to transparency and high welfare standards.

EARA's role extends beyond advocacy; it provides critical coordination and resources to foster a unified approach to openness. By facilitating communication between researchers, institutions, and the public, EARA has helped establish a culture of transparency that goes beyond compliance. The organisation also enables knowledge-sharing among signatories, fostering collaboration and solidarity to improve public understanding and trust in science and researchers.

Through these efforts, TAs have become a cornerstone for communicating the necessity of animal studies and the rigorous welfare standards underpinning them. They empower researchers to share their work confidently, contribute to public education, and promote policies that balance innovation with ethical considerations. By demonstrating openness and engaging in a dialogue with the public, individual institutions can then bring about a greater understanding of the reasons why animals are needed for research.

In supporting these agreements and continuing to advocate for transparency, EARA has significantly shaped a more open, informed, and trust-based relationship between science and society.

S3D2.2

Portugal's Path to Transparency: How Openness Enhances Ethical Standards and Promotes Culture of Care

A.I. Santos[1], I. Serrenho[2] and K. Leech[2]
[1]*NOVA Medical School, Lisbon, Portugal*
[2]*European Animal Research Association, London, United Kingdom*

Abstract
The Transparency Agreement (TA) on Animal Research in Portugal, launched in 2018 as a collaborative effort between Portuguese scientific organisations and EARA, was the third national agreement in Europe. Since its implementation, the Portuguese TA has grown significantly, encompassing more than 3 times the initial number of members, including research

institutions, universities, the pharmaceutical industry, scientific associations and patient organizations. All signatories commit to be open about the use of animals, improve communication, make proactive efforts to inform the public about animal research, and annual progress reporting.

This collective push for openness has resulted in tangible changes within member organizations. Researchers have now received communication training, enhancing their capacity for public interaction about animal research. Institutions are increasingly organising actions and events centered on transparency.

Signatories have demonstrated real progress, showing greater openness and transparency in their communication with the public. Many signatories regularly share images, videos, and information about their use of animals on social media channels and most have a position statement on their institutional website. The Portuguese TA also fostered a more compassionate and ethical research environment. Beyond meeting legal requirements, many members have adopted welfare practices that exceed regulatory standards, reflecting a deeper commitment to a culture of care. This initiative has built up collaboration, trust, and innovation among institutions and the public by promoting transparency, open dialogue, and the sharing of best practices. As a case study, this presentation will highlight the EARA-coordinated Patient Discovery project implemented in collaboration with members of the Portuguese TA.

S3D2.3

Balancing Ambition and Reality in 3R policy: Challenges and Opportunities in the Netherlands

M. Havermans[1,2], I. Serrenho[1], B. Tolliday[1] and K. Leech[1]
[1]*European Animal Research Association, London, United Kingdom*
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Abstract
The Netherlands has set ambitious goals for replacing animal research, often driven by the belief that a radical solution – even beyond what is immediately achievable – will accelerate progress without adverse consequences. But is this a valid assumption? There is a commonly heard narrative that alternatives to animal experiments are just around the corner and that phasing out animal research is possible as long as there is sufficient willpower.

However, such optimism can impact ongoing research where animals remain essential for answering critical scientific questions. It also raises concerns among researchers about continued effective implementation of the remaining two Rs – Reduction and Refinement - and it shapes the current public and political discourse in potentially counterproductive ways. What does not help is the serious lack of public understanding about the role of animals in research, which we see in The Netherlands and many other countries.

Therefore, the Dutch Transparency Agreement on Animal Research was launched in 2021, which brings together researchers and communication experts across the country to collaborate on openness and transparency. This presentation outlines the challenges Dutch researchers and science communicators face when navigating the public and political arena as well as their strategies, with the purpose of fostering a balanced and meaningful dialogue on a balanced 3R policy in the Netherlands.

S3D2.4

How Sharing Balanced Information Builds Trust in Science: A UK Concordat on Openness Perspective

H. Hobson[1]
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Abstract
Communicating how, when and why animal research happens allows people with no prior knowledge of the subject to have all the necessary information to feel more informed. This is why providing balanced information addressing both the harms and benefits of using animals in research is a core principle of the UK's Concordat on Openness.

Public audiences realise that animals are harmed in the pursuit of scientific knowledge and find the research easier to understand, and more credible, when this information is presented to them clearly. Getting the balance right is one of the most challenging aspects of openness, but it's important that organisations tell the story of their research and the expected benefits it will bring to society, while being clear about the level of harm and suffering the research entails.

UK signatories are providing balanced information through the publication of their animal use statistics. This includes the number of procedures or animals used per species, broken down via severity, with examples of experiments. Before the development of the UK's Concordat no signatories were proactively publishing these data. 11 years since its launch, this is now common practice.

Signatories are also publishing images and videos of animals undergoing procedures, research summaries that describe the animals' experience, and examples of how the 3Rs are used to mitigate suffering. To build public trust in science, accountability is key and presenting a more balanced picture of research by talking about what the animals experience, alongside study outcomes, is necessary for this mission.

S3E1.1

Empowering Scientists and Techs to Talk Confidently about Animal Research on Social Media

M. Harvie[1]
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Abstract
Are you tired of seeing animal research portrayed as cruel and unnecessary but don't know how to change the narrative? Would you like to talk about your work online but aren't sure where to start?

At UAR we've been tackling misinformation through social media for more than a decade but, we need your help!

The public wants to know how and why animals are used in science and how it benefits society at large, which is why we work with organisations in the animal research sector to communicate their work publicly.

As scientists, animal technicians, and communicators within the scientific sector your voice is critical to ensuring that animal research is understood and accepted by the public.

In this lecture, we will share our beginner-friendly tips for talking about animal research online, focusing on messaging, language, and accessibility. We will equip you with the skills to speak confidently about your work, including addressing severity and harms in animal research and communicating the animal's lived experience. We will also share with you our favourite (and free) tools for creating engaging and educational content for use online, and show you some of the resources we have created with those tools.

For many, their main concern around social media activities is the fear of negative backlash and managing comments, which is why we will also share our tried and tested methods for preventing and managing negative comments on social media.

S3E1.2

Supporting Replacement in Academia: Exploring Barriers Around the Acceptance and Uptake of Non-animal Methods

B. Reed[1]
[1]*RSPCA, Horsham, United Kingdom*

Abstract
In the UK (and in many other countries) more than half of all animal use for scientific purposes takes place within universities and medical schools, primarily in fundamental and applied research. There are particular barriers to changing current research practices in these settings. These include social and cultural issues relating to 'the way science is done', and established systems of funding, publishing and career progression which can incentivise or 'lock-in' certain behaviours.

This presentation will discuss an ongoing RSPCA project exploring how these factors may impact upon the ability of scientists across the range of career levels to train in and adopt non-animal methods. Drawing on findings from in-depth semi-structured interviews with researchers using animals across UK universities, this presentation will highlight drivers of animal use and barriers and opportunities around the uptake and acceptance of non-animal methods in academic research. Individuals shared their insights, perspectives and concerns as they spoke candidly about the issues they faced (including knowledge, skills, and expertise; communities and networks; academic environment and pressures; access; and awareness and engagement) and the support they felt they, and those like them, would need. In examining these factors, the project aims to offer tailored recommendations (e.g. to funders, regulators, professional and learned societies, journal publishers, Animal Welfare Bodies, individual institutions etc) for further stimulating and supporting the replacement of animal use in academia.

S3E1.3

The Animal Research Conversation: How Talking in Schools Can Change Beliefs about Animal Research

W. Jarrett[1]
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Abstract
Understanding Animal Research (UAR) has run The Animal Research Conversation, a programme of talks in UK schools, for more than 15 years. In that time, we have encouraged, trained and supported many researchers, veterinarians and animal care staff to discuss why and how they work with animals used in science, and answer questions from school children. UAR staff members have also given talks and run workshops in UK schools as part of this programme, sometimes debating with representatives of organisations opposed to the use of animals in research. Over the years, we have engaged with more than 100,000 young people.

The Animal Research Conversation is a two-way dialogue and allows young people the opportunity to explore their own beliefs about animal research and how animals used in science are treated. We track those beliefs and opinions before and after each session, so we know that finding out more about the reality of animal research helps young people to understand and accept the need for this research.

This presentation will demonstrate how young people's opinions change when they are given the chance to discuss this topic with experts and find out for themselves what happens inside a facility.

S3E1.4

Advancing Laboratory Animal Science Education: Virtual Reality for Practical Skill Training in FELASA Courses

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Abstract
Virtual reality (VR) has emerged as a transformative technology with promising applications across various fields, including laboratory animal science education. Traditionally, trainings in laboratory animal science include a theoretical transfer of knowledge and a practical skill training using live animals. Skill trainings are often offered species-specific but not solely task-specific addressing participants' particular needs in their experiments.

Considering the 3Rs, VR-teaching/learning units could help reduce animal numbers used for education by implementing them in existing courses, conveying practical skills virtually before using live animals. A shift from a species-specific to a more task-specific concept could further reduce the number of animals used in education.

Therefore, VR-teaching/learning modules were implemented into the practical skill training of FELASA Function A & D courses, where practical skills are also trained on live animals. As a pilot, 360° videos for mouse skill training were recorded, and 16 VR teaching/learning modules were provided to the participants before or after the skill training on live animals. Using an online evaluation tool, participants were asked to evaluate the quality, usability, and potential compliance with the 3Rs before and after engaging with the VR teaching/learning modules.

With the VR teaching and learning modules, the 3Rs have been further integrated into our teaching and learning concept for the laboratory animal science courses. This integration aims to provide more animal and task-specific qualifications for participants in the future.

S3E1.5

2018 - 2022 Implementation Report under Directive 2010/63/EU – Are we making progress?

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[2]*Dept. of Health, Northern Ireland, Belfast, United Kingdom*
[3]*European Commission, DG Environment, Brussels, Belgium*

Abstract
One of the main reasons for revising Directive 86/609 was to promote a level playing field for the scientific community across the Union.

The objective of implementation reports is to assess the progress, identify obstacles, and provide evidence base for improvement. The first Report covering the years 2013–2017 identified a several areas where the implementation was still evolving making it difficult to draw conclusions over the benefits and difficulties reported from Member States.

This presentation will highlight the differences between the first two reports, show where progress has been made in harmonisation, and explore the areas where significant differences and challenges remain.

The differing ways in which Member States have allocated responsibilities to Competent Authorities may lead to difficulties in ensuring an effective and consistent approach across a Member State.

There are also significant differences in the time taken to authorise projects which may negatively impact on scientific competitiveness, but different methods of determining this time may be contributory.

There is also in some Member States a reluctance for mutual acceptance of education and training undertaken in another Member State.

On a positive note, there are many areas where consistency has advanced at pace. One of the great successes has been the evolution of the Animal Welfare Bodies in promoting the Three Rs, especially in projects. There has also been progress in transparency with publication and improvement in the content of non-technical summaries.

Additional refinements to data collection for the 2027 report should further enhance the quality of data.

S3E2.1

Why a EU Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments

K. Schutte[1]
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Abstract
In its reply to the European Citizens' Initiative "Safe cruelty-free cosmetics – Commit to a Europe without animal testing" (Communication C(2023) 5041), the Commission committed to the development of a roadmap that will outline milestones and specific actions, to be implemented in the short to longer term, to reduce animal testing and that would be pre-requisites for a transition towards an animal-free regulatory system under relevant pieces of chemical legislation (e.g., REACH, Biocidal Product Regulation, Plant Protection Products Regulation and human and veterinary medicines). A similar roadmap had also been requested by the European Parliament in their resolution from 2021, asking for actions to accelerate a transition to innovation without the use of animals in research, regulatory testing and education.

The Commission now set out to collaborate with all stakeholders to develop such a roadmap by early 2026. Core of the roadmap will be to analyse and to describe the necessary steps to replace animal testing in pieces of legislation that currently require animal testing for chemical safety assessments. The roadmap will outline the path to expand and accelerate the development, validation and implementation of non-animal methods as well as means to facilitate their uptake across legislations.

The Commission has formed three working groups consisting of experts from Commission services, regulatory agencies (ECHA, EFSA and EMA) and targeted stakeholders from industry and academia involved in activities to identify non-animal replacement options for chemical safety testing and to develop a new framework allowing assessments without reliance on animal methods.

S3E2.2

Important Milestones in the Roadmap for Replacing Animal Testing for Industrial Chemicals

T. Sobanski[1]
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Abstract
Until recently, NAMs development aimed to fully replace animal testing for each specific regulatory endpoint. These developments have been successful for some relatively simple endpoints (like skin sensitisation), where the adverse effect and the mechanism(s)

leading to this effect are relatively well understood. Development of NAMs for more complex endpoints has so far been less successful.

By now, the scientific community and regulators widely accept that it would be almost impossible to develop one-to-one replacements of animal tests by NAMs for more complex endpoints such as e.g., repeated dose toxicity or reproductive/developmental toxicity. To identify and characterise the adverse effects underlying these complex endpoints, NAMs derived information should:

allow a conclusive outcome on the (lack of) hazardous properties for given regulatory endpoint: the conclusion should be scientifically sound;

reliably identify hazard and derive reference values to set safety levels, to communicate the hazard and assess the risks;

and reliably inform on the severity of the effect

In the presentation, a three-step-approach proposed by the European Chemicals Agency (ECHA) will be presented. In addition, some examples of short- to mid- term actions will be presented.

S3E2.3

EPAA Collaboration in the Development of the EU Roadmap

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Abstract
The European Partnership for Alternative Approaches to Animal Testing (EPAA) is a unique partnership between the European Commission (DGs GROW, ENV, SANTE, JRC, RTD), European Regulatory Agencies (ECHA, EFSA, EMA), European Industry Federations (AISE, AHE, CEFIC, CE, CLE, EFPIA, FDE, IFRA, SMEUnited) and 39 companies to refine, reduce and replace regulatory animal testing.

In recent years the EPAA has re-focussed its activities to align with and inform the European Commission Roadmap towards phasing out animal testing for chemical safety assessment through establishing a series of platforms to co-create proposals on how to address strategic roadmap challenges:

- Use of New Approach Methodologies (NAMs) for Human Health Systemic Safety Assessment via EPAA NAM Designathon Challenge & EPAA NAM User Forum
- Use of NAMs for Environmental Safety Assessment, following the 2023 EPAA Partners Forum and new EPAA project on this topic
- Use of NAMs for the Assessment of Endocrine Disruption, via 2024 EPAA Partners Forum

In addition, EPAA has initiated new project activities to develop roadmap proposals to address priority regulatory needs on Use of NAMs for Acute Toxicity Assessment, Second-Species Sub-Chronic Toxicity Testing and implementation of a new paradigm for Carcinogenicity & Genotoxicity Assessment. Finally, in March 2025, EPAA will partner with the European Commission to organise a multi-stakeholder conference on Animal-Free Chemical Safety Assessment to develop, discuss and refine draft Roadmap and Test Method & Validation strategy proposals.

S3E3.1

Facility Construction and Reconstruction Challenges

J. Honetschläger[1]
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Abstract
The construction and reconstruction of animal facilities present unique challenges that require careful planning and execution. This presentation aims to share with the audience some issues they can face during construction processes, which are critical to avoid problems when running the animal facility later. More complicated issues can arise when reconstruction or repairs are needed in an already-running animal facility, where animal anxiety, stress, and biased experiments also have to be considered. Practical examples will highlight the importance of attending control days, managing unexpected situations, and ensuring proper sealing and noise control. The aim is to provide insights into overcoming these challenges and ensuring animal facility projects' successful completion and operation.

S3E3.2

Leadership and Team Management: Creating Excellence in Laboratory Animal Science

M. Kamper[1]
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Abstract
Effective team management and leadership are crucial components in maintaining high-quality laboratory animal facilities and ensuring optimal animal welfare. This presentation explores key strategies and practical approaches for building and managing successful teams in laboratory animal science facilities. Drawing from real-world experiences, we examine three essential pillars of effective facility management: team structure and dynamics, communication frameworks, and professional development initiatives.

The discussion focuses on evidence-based management techniques specifically adapted for the unique challenges faced in laboratory animal facilities. We present case studies highlighting successful team transformations, including the implementation of structured feedback systems, cross-training programs, and conflict resolution protocols. Special attention is given to managing diverse teams with varying levels of expertise, from animal care technicians to veterinary specialists.

The presentation also addresses common management challenges, featuring examples of both successful interventions and learning opportunities from less favorable outcomes. These real-world scenarios demonstrate how effective leadership strategies can overcome obstacles such as staff turnover, communication barriers, and resistance to change. We explore practical solutions for fostering a culture of continuous improvement, maintaining team motivation, and ensuring consistent adherence to animal welfare standards.

Participants will gain insights into developing robust team management frameworks that promote excellence in laboratory animal care while maintaining high staff satisfaction and retention rates. The discussion includes practical tools for performance evaluation, team building exercises, and strategies.

S3E3.3

Care and Welfare of Animals

N. Dennison[1]
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Abstract
Facility staff and researchers face frequent challenges in trying to optimise animal care and welfare in research facilities.

Good habituation and positive reinforcement training can allow improved approaches to human-animal interactions and procedures by reducing stress for both the animals and the staff involved. Setting up a 3Rs champions group has allowed technicians to lead on practical approaches to this and successful implementation of voluntary oral dosing as a refinement to oral gavage, as well as providing information on better ways to support mouse pups around weaning. An example of the challenges and successes for improving conditions for breeding rats by improving social interactions, space and enrichment practices, whilst also improving numbers of pups born and so allowing reduction in the numbers of breeding adults required will be given.

Facility staff working closely with researchers allows development of improved assessment of clinical phenotypes of genetically altered mouse strains and better approaches to welfare scoring on studies. An example of how collaboration can benefit both animal welfare and improve research outcomes relating to pain monitoring in an arthritis model will be given. It is particularly challenging to develop good humane endpoints for phenotypes that worsen slowly over time, but good communication of concerns and scientific requirements is instrumental in doing this successfully. This will be discussed.

S3E4.1

Working Together to Reduce the Environmental Impact of Laboratory Animal Science

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Abstract
The AFSTAL Sustainability Working Group is constituted of seven members with a wide range of responsibilities across our community. We work together with a single common goal in mind: to reduce the environmental impact of laboratory animal science. Our first step was to run an international survey on environmental impacts and laboratory animal facilities. With over 300 respondents, it gave us a snapshot of the topics of concerns amongst participants. We then gave three workshops at the AFSTAL congress, so that attendees could discuss how to estimate carbon footprints, how to reduce waste and consumable consumption, and how to reduce the environmental impact of husbandry and biosecurity. Here, we will summarise the results of the survey and highlight the main points of these three major topics. For example, we will discuss the role of facility management to reduce the consumption of Personal Protective Equipment, to adapt ventilation, and to space cage changes. However, the main message is that the challenge does not have a simple and easy solution. It has many routes to explore. The next mission of the working group is to identify how to work together with the community. We are trying various initiatives, and we will present the main results. The aim is for the audience to leave with many new ideas to test, networks to consult, and the ability to use tools enabling the reduction of the environmental impact of their animal facilities.

S3E4.2

The Importance of the Microbiome and Genetic Variables in Research Reproducibility

S. Lawton[1]
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Abstract
Thorough biological characterization of animals is critical to ensuring that research observes the principles of the 3Rs. Decades of research have demonstrated that the microbiome is an essential component of animal characterization, as changes in the microbiome can cause phenotypic differences within an experiment, or across experiments, which can lead to the misinterpretation of results. By accurately defining and monitoring the microbiome composition of strains, researchers can protect their experimental reproducibility and minimize outliers.

With substantial recent developments on the gut-brain axis, it has become apparent that the nervous system is one of the areas most impacted by changes in the microbiome. As sequencing developments have allowed microbiome analysis to become more accessible, they have also allowed more thorough genetic characterization of research models. For example. neurodegenerative models often contain short tandem repeats (STRs). These models are usually tested for the presence or absence of mutations, but the precise sizing of the length of these unstable regions has profound impacts on model severity. Further, expansion or contraction of the alleles can be driven by the sex of the breeders used in the colony, requiring ongoing monitoring to ensure the mutation lengths and resulting phenotypes remain consistent. In order to properly characterize research models, it is critical that animals have their health status monitored, background strain verified, genotypes and sizing confirmed, and microbiome composition established. When reported together, the transparency of these variables can help maximize research reproducibility.

S3E4.3

Managing an Animal Facility During Conflict: Lessons from the Technion, 2024

D. Mintz[1]
[1]Technion (Israel Institute of Technology), Haifa, Israel

Abstract
The ongoing war in Israel in 2024 has posed unprecedented challenges for maintaining operations at the Technion's animal facility. As a vital resource for biomedical research, ensuring the welfare of our animals while safeguarding staff and maintaining operational continuity has never been more critical. This presentation outlines the key strategies that we implemented amid these extreme conditions to navigate a complex crisis.

We will detail our crisis management protocols that were swiftly activated to address immediate threats and long-term operational concerns. This included the establishment of remote monitoring systems, enabling us to oversee animal care and facility conditions even during lockdowns and evacuations. Additionally, our comprehensive emergency contingency plans were pivotal in ensuring that both animal welfare and research integrity were upheld, in strict compliance with AAALAC standards.

Through this experience, we gained valuable insights that extend beyond our specific situation. We will emphasize the importance of robust planning, flexibility, and communication in crisis scenarios, as well as the need for facilities to proactively cultivate a culture of preparedness. This presentation aims to provide actionable strategies for other animal research facilities facing similar challenges, regardless of their scale. Together, we can learn from these experiences to enhance our resilience and commitment to ethical research practices during adversity.

S3E4.4

Neuroinflammation Persists for at Least One Year After a SARS-CoV-2 Infection in Macaques

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Abstract
Neurological symptoms associated with SARS-CoV-2 have drawn significant attention since the onset of the pandemic. The mechanisms underlying the symptoms remain poorly understood and it is unclear if it results from direct effects of the virus or from indirect effects caused by disturbances in the immune system. Imaging the outer mitochondrial membrane translocator protein (TSPO) provides a means of assessing neuroinflammation *in vivo*.

To investigate neuroinflammatory processes longitudinally after SARS-CoV-2 infection, four SARS-CoV-2-infected rhesus macaques were monitored for a year with positron emission tomography-computed tomography (PET-CT) using [18F]DPA714 as a tracer for TSPO. A region of interest over the entire brain was quantitatively analyzed by which the baseline PET-CT was compared to PET-CTs obtained post-infection.

The PET-CTs revealed an increased tracer uptake throughout the brain in 3/4 animals. In two animals the uptake peaked at 6 months post-infection (16–32%) while the uptake at 12 months post-infection was still above baseline values (7–19%). The third animal showed also an increased uptake at 6 months (11%) but this increased even further at 12 months (24%). Next to the PET-CTs also plasma NfL, a validated biomarker for neurodegeneration, was analysed. NfL peaked at two weeks post-infection. Nevertheless, a year after infection these levels were, in all animals, still significantly increased compared to pre-infection levels (128% range: 70–179%).

This study implicates persistent neuroinflammation following a SARS-CoV-2 infection, like longCOVID patients1, for at least one year in macaques which is not focused on a specific brain region but can be observed widespread throughout the brain.

S3E4.5

Unite and Conquer: Strategic Roadmap for Integrating and Advancing Two Rodent Facilities

I. Moreira[1]
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Abstract
The Gulbenkian Institute for Molecular Medicine (GIMM) is a leading research institution in Portugal, driving groundbreaking discoveries and innovative healthcare solutions. Formed through the merger of two prominent institutes, GIMM exemplifies the power of unification in advancing scientific progress.

As part of this institutional merger, integrating two rodent facilities with distinct operational models is a complex process. This transition involves consolidating resources to support facilities with a combined capacity for 10,000 cages, 30 team members, 255 users, 44 research units, and 49 projects. The merger strategy outlines a five-year roadmap for success.

A comprehensive assessment of both sites led to the creation of a Business Model Canvas, which shapes the strategic vision and guides decision-making. The integration strategy is based on four key pillars:

Operations: Enhancing efficiency and optimizing resources.

Strategic Integration: Unifying processes and cultivating a shared culture.

Building the Team: Strengthening collaboration and internal capacity.

Growth and Innovation: Identifying opportunities for expansion while aligning with strategic goals.

Early results highlight improvements in animal welfare, streamlined operations, and enhanced research support, fostering a culture of care and collaboration.

By the time of the FELASA 2025 Congress, two full trimesters of the merger will have been completed. This presentation will provide a candid overview of successes, challenges, and mid-course adjustments, illustrating how the merger aligns with the broader goal of advancing laboratory animal science. Our experience demonstrates the potential of strategic facility planning to drive meaningful change in the field.

S3F1.1

Involvement of Animal Caretakers and Technicians in the Planning and Conduction of Animal Experiments

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[2]TARCforce3R Center, University Medical Center, Mainz, Germany

Abstract
Animal experiments are crucial for understanding complex biological systems and therefore, for the advancement of various scientific fields like medicine or agronomy. Central to the quality of such in vivo research are an appropriate study design and the compliance to strict protocols. However, the impact of animal caretakers and laboratory technicians on the planning and realization of animal studies is often overseen.

To evaluate the involvement of caretakers and technicians in animal experiments, an anonymous, explorative online questionnaire study was conducted. The survey was designed to capture both quantitative and qualitative data from principal investigators, veterinarians, animal caretakers and laboratory technicians all over Europe.

Overall, 568 answers from researchers and 849 answers from non-scientific staff from more than 30 different countries could be collected. From the researchers' perspective, communication with all people involved in the project was good. Animal caretaker and laboratory technicians are the group mostly involved in conducting the study, whereas mostly authorities, animal facility manager and laboratory technicians are involved in planning of the study. Animal care staff and laboratory technicians stated that they agree to be "involved in the discussion about planning and conducting experiments" and "trained on experimental planning and protocol elaboration", both serving to understand why and how each experiment is done.

In summary, researcher and nonscientific staff are willing to communicate regarding planning and conduction of animal studies and see it as useful for their projects. Nevertheless, the frequency of actual discussions about planning of the experiments is low.

S3F1.2

The Quality of Animal Research Depends on All Active Players

B. Bert[1], C. Heinl[1] and F. Gonzalez Uarquin[2]
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[2]TARCforce3R Center, University Medical Center Mainz, Mainz, Germany

Abstract
Basic and translational biomedical research still relay on animal experiments and provide the basis for clinical trials. This places an increased duty of care on researchers to derive the maximum possible output from every experiment in the best quality. However, experimental studies are often poorly planned, involve questionable research practices like HARKing (hypothesizing after results are known) and p-hacking, and results are selectively reported, which all strongly impair the validity of published data. Moreover, recent studies suggest that a large proportion of animal experiments are actually never published. These practices not only slow down the scientific progress they also represent an ethical issue if animal lives are wasted without bringing any knowledge gain.

Open science practices like preregistration, data sharing and reporting of negative results have the great potential to initiate a change in research culture. Their implementation throughout the whole research process, from study design, through data collection and analysis, to publication and dissemination can improve transparency and quality of translational research in general.

A successful implementation of these practices requires that all persons involved in the scientific experiment have the relevant knowledge. For example, lab technicians have to understand why blinding and randomisation is important for receiving unbiased data. On the other hand, researchers can learn from animal care takers important facts about the animal behavior or biology which are important for planning the experiments. This entails good communication between all persons involved in the animal experiment. Our survey reveals that there is still room for improvement.

S3F1.3

The Role of Technicians in Qualitative Assessment of Animal Welfare and its Subjective Experience

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Abstract
The concept and practice of animal welfare is rooted in the idea that for each species an objective list of indicators (physiological as well as behavioral ones) can be provided to assess and promote the individual state of welfare. Nonetheless, each individual is characterized by personality traits, and therefore, a qualitative dimension of animal welfare escaping from a list of objective indicators can exist. The case of Non-Human Primates seems to be particularly meaningful to explore this qualitative dimension of animal welfare. In this pilot study we aimed at sketching an analysis of the qualitative dimension of animal welfare through interviews with technicians working with NHPs. The goal was also to investigate the role technicians can have in improving the quality of life of NHP used in laboratory research. We interviewed a sample of technicians working in basic and regulatory research, asking question related to the assessment and actions to be taken for improving the welfare of NHP in their care. The overlap between physiological and psychological wellbeing, the importance of temperament and individuality, and the importance of the bond and mutual trust established between the experimental monkeys and the staff will be discussed. Finally, as pointed out by all of the interviewee, the value of an empathic attitude in experimental work with animals will be emphasized.

S3F2.1

Why Learning Outcomes for Competence Assessors

K. Schutte[1]
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Abstract
The Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes developed a common education framework[1] to facilitate meeting the requirements for competence of all those involved in the use and care of animals for scientific purposes and the free movement of personnel across the Union.

In order to avoid problems related to inappropriate animal handling, proper training of personnel is as important as the assessment of personnel demonstrating the requisite competence in practice. In addition, individuals shall maintain their competence through regular review and updating practices as necessary by means of continuing education (Continuing Professional Development – CPD).

To ensure a harmonised approach to this, facilities should have a robust framework within which training and supervision takes place, with clear standards defining competence in knowledge-based and practical skills. The learning outcomes for competence assessors aid in applying harmonised standards and promote a consistent approach to the assessment of the competence of all personnel across facilities in all of EU-27 and Norway.

Learning outcomes for competence assessors aid in harmonising what standards (e.g. pass/fail criteria) will be applied to an individual's assessment. An assessment methodology should be used to guarantee objectivity (e.g. by using detailed DOPs [Direct Observation of Procedural Skills). This allows the process to be objective, and therefore consistency across competence assessors can be achieved.

S3F2.2

Education, Training, and Competence in Laboratory Animal Science

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Abstract
Ensuring competence in laboratory animal science (LAS) relies on effective education, rigorous training, structured and validated assessment methods, and continued professional development (CPD). Competence in LAS extends beyond knowledge and technical skills to include ethical and professional conduct. Current best practices emphasize species-specific training, modular education and training frameworks, and blended learning approaches integrating synchronous and asynchronous methods. Formative and summative practical assessments of skills such as Direct Observation of Procedural Skills (DOPS) are essential for certifying readiness to work independently with live animals. Periodic competence reviews and CPD activities are imperative for maintaining high standards in LAS. This framework safeguards animal welfare and promotes scientific quality, integrity, and public trust in animal research.

S3F2.3

Learning Outcomes for a Competence Assessor

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[3]Universidade do Porto, i3S, Porto, Portugal

Abstract
The humane treatment of animals used in science entails that they are used and cared for by competent staff. Competence can be achieved through education and training, experience, and continuing professional development1. The education and training framework to fulfil the requirements under the Directive 2010/63/EU[2], states that competence grows on the job, and establishing this growth can be done in five steps. With each step the supervision needed is reduced until the best level is achieved, in which one can be entrusted to work fully independently. To establish trust in an activity with animals is not an easy task, for it needs clarification that the candidate assessed has the appropriate knowledge, skills, attitude and empathic behaviour towards animals. To evaluate the latter, in particular, it is best to directly observe candidates in practice. However, due to this difficulty in assessing entrustment within – and as a consequence of – training, there is a need for adequately training staff in competence assessment on the job. Therefore, the European Commission instructed the establishment of learning outcomes for training competence assessors. According to these outcomes, assessors should be trained to: understand the importance of training and assessment; develop and perform assessments; enhance and establish trust; and reflect on the quality of the assessment process. These are inspired by medical education where entrustable professional activities are becoming key components of establishing competency of staff involved in patient care3, a development which can now enhance the care for animals used in science.

S3F2.4

Training Competence Assessors

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[2]Animal Welfare Body, Utrecht, Netherlands

Abstract
Training and assessment of laboratory animal science professionals are critical for maintaining high standards of animal welfare and scientific reliability. A blended learning course has been developed to equip supervisors and assessors with the necessary skills to evaluate and enhance competence in experimental procedures. Through guided peer discussions, collaborative

assessment exercises, and instructor-led sessions, participants are taught to create a safe and empathetic environment. These activities encourage critical reflection, mutual learning, and confidence in assessing tasks. The program combines online modules with practical workshops to ensure knowledge and expertise. Fundamental concepts are introduced through the online ETPLAS module about competence assessment, while in-person sessions aid in developing skills and attitude to achieve competence in assessment and supervision. Participants engage in exercises like designing Direct Observation of Practical Skills (DOPS) and delivering feedback using structured frameworks. The course's format allows a gradual and systematic approach, starting with understanding assessment objectives and culminating in the execution of well-founded evaluations. Each session builds upon the previous one, incorporating preparatory tasks, interactive learning activities, and reflections. Role-play scenarios, video assessments, and portfolio development are integral components, leading to an end-assessment that validates participants' competence. Programmatic assessment ensures that testing aligns with the training's objectives, offering a comprehensive evaluation of participants' progress. This training enhances professional capabilities of supervisors and assessors and further contributes to improved animal welfare and more reliable research outcomes. By emphasizing interaction, blended learning, and structured lesson plans, the course establishes a standard for competence-based training in laboratory animal science.

S4A1.1

Implementing Directive 2010/63 on Genetically Altered Animals in France: Lessons from Experience

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Abstract
When the 2010 directive was published, the provisions regarding the use of genetically altered animals were open to interpretation. With the adoption of the Commission Implementing Decision (2020/569) in 2020 and the update of the European guide on genetically altered (GA) animals in 2022, that complete and concrete guidance became available.

In France, we prioritized this issue, recognizing its impact on statistical reporting and the need for practical solutions. To address these challenges, we developed several tools. First, we produced a simplified note and a guide tailored to the needs of French establishments. As part of our ongoing commitment to clarity and support, we organized a webinar-recorded and made accessible to all and participated in various national seminars and conferences on the subject since 2022.

Simultaneously, we encouraged establishments to progressively align with the regulations concerning the use of GA animals during 2022 and 2023, particularly for project applications and statistical reporting. Today, we are witnessing clear progress in project submissions, the use of GA animals, and statistical accuracy.

Building on this robust regulatory foundation, we are now better equipped to focus on improving animal welfare for genetically altered animals. This includes advancements in tissue sampling methods for genotyping and improved management of harmful phenotypes.

S4A1.2

Reporting on the Creation, Breeding and Use of Genetically Altered Animals under Directive 2010/63/EU

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Abstract
The creation and maintenance of genetically altered animals (GAA) were explicitly brought within the definition of a procedure under Directive 2010/63/EU, where such animals may experience a level of pain, suffering, distress, or lasting harm, equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

The early days were marked by a degree of ambiguity and confusion on several topics including how to consider Genetically Altered Animals (GAAs) within projects and assign appropriate severities. To address this, initial GAA guidance was endorsed by Member States in 2013. Nonetheless, issues such as reporting of GAAs (annual statistics and within 5-year implementation reports) have remained challenging, and a revised, comprehensive guidance was issued in 2021.

The use of GAA is characterised by rapid technological advancements and there is new evidence of species capacity to experience pain, distress, and suffering. Additionally, GAA creation and maintenance are significant source of annual surplus, as shown by the 2017 and 2022 Implementation reports.

The presentation will outline the trends in the use of GAAs under the Directive and address remaining challenges such as correct assignment of phenotypes (harmful vs non-harmful), their handling within a project application and related reporting obligations. It will also explore opportunities to reduce surplus.

S4A1.3

To Be or Not To Be. . .Harmful: The Dilemma within Welfare Assessment of GM Mice

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Abstract
Directive 2010/63, Article 4.3, mandates Member States to ensure the refinement of breeding, accommodation, and care, as well as methods used in procedures, to minimize any pain, suffering, distress, or lasting harm to animals. Despite significant efforts to clarify aspects of harm over the years, grey areas remain.

This highlights the need for consensus on assessing what constitutes harmful phenotypes, as classifying a phenotype as harmful or non-harmful has profound implications for animal welfare as well as the legal maintenance of a GA line, determining whether a project authorization is required.

As technologies for generating and characterizing Genetically Altered (GA) animals evolve, interpretations of harm may vary depending on scientific advancements and regulatory perspectives.

This presentation will explore these complexities, offering practical examples and introducing the ongoing activities of the FELASA Working Group on Harmful and Non-harmful Phenotypes of Mice (https://felasa.eu/working-groups/present/id/60). The presenter, who also serves as the Convenor of the Working Group, will share insights into their collective efforts to explore frameworks for evaluating harm and enhancing animal welfare, while fostering a more unified understanding of and approach to this challenging and nuanced topic.

S4A1.4

Different Humanised Mouse Models for Different Applications: Pros, Cons and Severity

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Abstract
In recent years there has been a proliferation in the use of humanised mouse models in biomedical research. The initial uptake of these models was driven by their use in oncology and virology applications but is now spreading into all areas of research. The plethora of different humanised models outlined in the literature means that identifying which humanised model to use for each application, the welfare implications of a particular model and how these can be mitigated to reduce severity can be difficult.

Here we outline:

- That a humanised model consists of the strain of immunocompromised animal used and the humanisation method.
- What the pros/cons and welfare implications of the humanised models are and
- The mitigation steps that can be implemented to reduce the severity seen in humanised mouse models including scoring systems and increased welfare checks.

S4A2.1

The EU Transparency Framework and its ALURES Databases

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Abstract
Compliance with legal requirements is a prerequisite for conducting research with live animals. Accountable to public, the research community is expected to provide transparent information on the uses of animals in science. This was echoed during the negotiations of the Union legislation on the protection of animals used for scientific purposes in 2010, which incorporated transparency measures into its legal text.

To provide valuable insights for policymakers, funders, and fellow researchers, data must be timely and detailed. Further measures were introduced just a few years later requiring the publication of raw data on animal use statistics and non-technical project summaries (NTS) through open access databases. These data are not only a matter of compliance, but also a powerful tool for driving progress with the Three Rs.

ALURES statistical database allows datamining, now at Member State level, to help understand animal use trends, the purposes and the uses resulting in severe suffering. Meanwhile, ALURES NTS database provides further insight into how animals are used and what obstacles there are for a wider implementation of Replacement, Reduction and Refinement.

The user interface of the NTS ALURES has recently been updated, incorporating innovative tools such as AI-powered search facilities, making it easier than ever to explore the data and uncover new insights. With some practical examples that follow this talk, we hope that more stakeholders will find their way to ALURES – to assess, analyse and take action!

S4A2.2

Use of ALURES Non-technical Summaries for improving the REPLACEMENT in Cardiovascular Disease Research

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Abstract
The EU ALURES database provides valuable insights into the current animal use in research and testing. Here, we present an analysis of Non-Technical Summaries (NTS) from authorised cardiovascular (CV) research projects involving animals, submitted to ALURES (Animal Use Reporting - EU System) between 2021 and 2023. The objective of this study is to build structured knowledge from the NTS to evaluate opportunities for replacing certain animal-based models with human-relevant, non-animal alternatives.

The NTS were classified based on several criteria: the investigated pathology, the species of animals involved in the projects, the predicted severity of harm inflicted to those animals, the animal's fate after the project, and the quality of the declared replacement strategies. The analysis of over 3000 NTS revealed that the most common project aim is the investigation of physiopathological mechanisms associated with specific diseases and that the majority of NTS are focused on heart failure. Notably, over a period of eight years, the NTS studies projected potentially more than 7 million animals from 31 different species.

Based on this knowledge, we aim to accelerate the adoption of non-animal methods, reducing animal use and enhancing

research outcomes by comparing how and why animals are used in cardiovascular diseases research with our catalogue of existing non-animal models.

Through this work, we seek to foster dialogue and collaboration within the cardiovascular research community, focusing on concrete and specific aspects of the biomedical research to promote the principles of the Three Rs (Replacement, Reduction, and Refinement).

S4A2.3

Using the ALURES Database for Developing Indicators to Monitor the Impact of EU Legislation

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Abstract
The European Union has enhanced transparency in animal research and testing through the open-access EU ALURES database. This dynamic platform provides continuously updated and harmonised data for online analysis. Beyond promoting transparency, this resource serves as a critical foundation for informed decision-making in research, funding allocation, and policy formulation.

By analysing data from the ALURES database, trends in animal use can be identified, highlighting opportunities to implement alternative approaches. In line with the EU Directive on the protection of animals used for scientific purposes, six statistical reports have been published, covering a period from 2015 to 2022. These reports show how various dimensions of the ALURES data can be combined to provide valuable insight into animal use. For instance, they showcase the adoption of newly developed replacement methods, identify areas requiring further improvement, and offer a clearer understanding of sectors utilising animals, particularly for research purposes.

More recently, the Commission used the statistical ALURES data to develop indicators for monitoring animal use in regulatory testing of industrial chemicals, assessing the effectiveness of the Union chemicals legislation in achieving its primary objectives.

Looking ahead, the next step involves creating additional indicators to measure progress toward phasing out animal testing for chemical safety assessments. This initiative aligns with the European Commission's response to a European Citizens' Initiative in 2023, which called for a concrete commitment to building a Europe without animal testing.

S4A3.1

Microbial Contamination of Biological Materials: A Risk for Animal Research and the Reproducibility Crisis

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Abstract
Microbial contamination of biological materials such as transplantable tumors and cell lines can pose significant risks to animal health, personnel safety, data integrity, and experimental reproducibility. Adventitious agents are a significant contributor to the widespread challenges in replicating results from both cell line-based research and in vivo studies. Moreover, since biological materials are often engrafted into severely immunodeficient mice, preventing the introduction of infectious agents via biological materials is a critical component of biosecurity for animal research facilities. Thus, the laboratory animal research community has an ethical responsibility to protect animal welfare and conduct biomedical research using methods and protocols that produce valid and reproducible results. This presentation will overview potential impacts of biological materials contaminated with infectious agents as well as discuss methods for detection and risk mitigation. The target audience includes veterinarians, facility managers, scientists and technical personnel who work with biological materials or manage colony health monitoring programs.

S4A3.2

AniMotion: "Role-play as Tool for Selecting the Most Suitable Animal Model in Animal Experimentation"

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Abstract
The choice of animal species for experimentation is a complex issue that requires multidimensional and interdisciplinary thinking. European animal welfare laws stipulate that the choice must be justifiable not only from a scientific but also from an ethical perspective. However, arriving at a holistic justification that satisfies the legal, scientific and ethical demands is challenging, as the demands often conflict. Addressing this problem, we have developed an innovative role-play game designed to foster awareness and accountability in the choice of animal species for experimentation. This game, developed by an interdisciplinary team of scientists, bioethicists, and legal scholars, enables participants to engage in immersive, in-depth discussions regarding the pros and cons of employing various animal species in experimental setups.

Through a dynamic and engaging session, participants are assigned roles embodying unique perspectives and encouraged

to play the game actively. The game involves 'case cards' representing different scenarios, facilitating prudential decision-making. By weighing the pros and cons of presented cases, participants select arguments that align with their assigned roles, promoting role-based weighting and a collaborative discussion of the game results.

The game aims to provide a practical understanding of decision-making in animal experimentation. It can help discuss responsibilities in scientific practice and explore its potential applications in various fields, including research, education, and policy. The game's output can serve as a rationale for the selected animal species in the application. By sharing our experiences with this innovative approach, we aim to contribute to a more informed and responsible discussion about animal experimentation.

S4A3.3

Contributions of Sex Bias to Animal Welfare: It's Everyone's Animal Welfare Problem

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Abstract
Sex bias is a common practice in biomedical research. Every year, there are approximately 12.5 million surplus mice and rats produced in the EU. Unfortunately, the vast majority of these animals are then euthanized, resulting is the ethical dilemma and animal welfare issue of animal wastage. Poor colony breeding management, overreliance on single sex (*i.e.* male rats and female mice), and reluctance to use modernized experimental design all collectively contribute to significant animal wastage. While there is significant interest in the laboratory animal community and amongst regulatory oversight bodies to reduce this surplus animal production, minimal progress has been made on this problem. We continue to see an overreliance on male rats and female mice in published research, and large depopulation events continue to occur in vivaria around the globe as surplus animals are culled. This session will summarize how common research practices and experimental designs have fueled the sex bias issue leading to this sizable surplus. We will focus on the contribution of sex bias to animal overproduction at both the level of the commercial producer, as well as the individual investigator laboratories. We will also discuss practical solutions, including appropriate experimental design and colony management tools, to help decrease animal wastage and improve animal welfare.

S4A3.4

Improving Laboratory Animal Genetic Reporting: New Guidelines

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Abstract
The biomedical research community acknowledges the challenges of reproducibility in research and has initiated efforts to address this issue. These encompass nomenclature guidelines, enhancement and pre-registration of experimental designs, and open access initiatives. Crucial to this is the establishment of common documentation standards for animal experiments. The ARRIVE guidelines outline key aspects for documenting laboratory animals used in experiments, but a need remains for more comprehensive information on the genetics of research animals.

We brought together a large number of leading international experts, consortia, and learned societies in the field of animal genetics to define the Laboratory Animal Genetic Reporting (LAG-R) recommendations. The recommendations have been published in Nature Communications. This LAG-R framework defines a set of guidelines to support more complete documentation of the genetic make-up of animals used in research.

The purpose of LAG-R is to help authors better describe the genetics of laboratory animals in scientific papers, but also to provide editors and reviewers with a checklist of essential genetic information that needs to be included in a publication. LAG-R provides an easy-to-understand list of recommendations together with information on available resources to support the use of standardized nomenclatures with the goal of improving reproducibility, reliability, and overall scientific rigor.

S4A3.5

Refinement of Metabolic Cage Design to Enhance Animal Welfare

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Abstract
Metabolic cages are commonly used in laboratory animal research—particularly with rodent models—because they enable extended monitoring of individual animals and facilitate the separate collection of urine and feces. However, the conventional design of these cages can induce stress through isolation, restricted movement, minimal environmental enrichment, and high light exposure. This heightened stress compromises both animal welfare and the quality of experimental data.

In our recent study, we refined standard metabolic cages to enhance animal welfare without compromising data-collection feasibility. We repositioned the feed racks so each animal had improved visual and olfactory contact—and remained as close as possible—to neighboring cages during feeding. A dark shelter was placed in each cage for enrichment, and we reduced light levels by darkening parts of the cage environment. In addition, the entire rack holding four metabolic cages was covered to further minimize both light exposure and noise. 12 male Sprague-Dawley rats (body weight: 412 ± 28 g) were housed individually in these modified cages for 24 hours, and urinary IL-6 and corticosterone levels were compared with those of conventionally housed animals ($n = 6$).

Altogether, these refinements led to increased food intake, a trend toward lower urinary corticosterone, a significant reduction in urinary IL-6, and fewer stress-related behaviors. Importantly, these improvements are straightforward to implement and align with the 3Rs principle. Future work will focus on cage-specific handling training and additional enrichment strategies to further refine metabolic cage housing, thereby enhancing both animal well-being and the reliability of experimental results.

S4A4.1

Improvement in Non-technical Summary Information on Replacement to Improve Transparency

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Abstract
Public confidence that animal research is required in specified circumstances can only be facilitated if information provided in non-technical summaries (NTSs) is sufficiently detailed to demonstrate that there really is no alternative option than the use of live animals. Currently, there is evidence that researchers are not explaining clearly and specifically what alternatives have been tried, or are actually being used in parallel. Furthermore, it is not always explained what alternative methods have been considered and/or why those methods will not suffice. It may not be clear that sufficient effort has been made to seek for alternative methods.

The quality of some NTSs in the ALURES database in respect of replacement section will be explored, and poor and good quality examples will be provided. Questions to answer and issues to cover will be proposed with the intention of assisting in improving submissions of future non-technical summaries to increase the likelihood of high quality submissions. These measures will improve the transparency of NTSs and improve public confidence. Such proposals will be of value to researchers, project evaluators, competent authorities for project authorisation, and any other person who contributes to the quality of the NTS.

S4A4.2

Systematic Review of European Public Attitudes Toward Animal Research: Trends and Implications

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Abstract
Public acceptance of animal research in scientific fields is significantly influenced by societal knowledge, beliefs, political

affiliation, and general opinions. To improve our communication practices, it's important to gather information about public opinion on what we are doing (and how).

Despite its importance, the last pan-European opinion poll asking specific questions about animal research was more than a decade ago (Eurobarometer Science and Technology, 2012). Additionally, there is a lack of systematic review of studies on public attitudes toward animal welfare and the use of animals in science, especially if used for studying human health and disease mechanisms. Thus, we systematically reviewed and analysed over 30 national or targeted polls and survey data to have an overview of public opinions and attitudes toward animal research while discussing trends, regional variations, and factors influencing public perceptions.

A systematic literature search was conducted across multiple databases, including academic journals, governmental reports, and reputable polling organisations, to identify that studies assessed public opinion on animal research. Preliminary findings suggest a complex landscape of public opinion, with varying levels of acceptance and concern influenced by factors such as cultural context, academic level, awareness of scientific practices, societal values, and cultural emphasis on animal welfare in general. Knowing public attitudes towards animal research is essential for developing effective communication strategies, guiding policy decisions, and addressing ethical considerations in biomedical and scientific research.

S4A4.3

Communicating Compliance, a Canadian Perspective: The University of British Columbia Post Approval Monitoring Program

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Abstract
Post Approval Monitoring (PAM) is an essential part of the UBC Animal Care and Use Program (ACUP), ensuring compliance with approved protocols and policies for all UBC Persons using animals in research, breeding, and/or teaching. The foundation of the UBC PAM program is the Canadian Council on Animal Care (CCAC) policies and guidelines, and UBC ACC Policies and related documents, which clearly establish the mandatory "must" versus the recommended "should" requirements. The UBC PAM program is multi-pronged involving the Animal Care Committee (ACC), PAM Team, and Animal Facility Management. The collaborative and educational nature of the UBC PAM Program results in a proactive versus reactive resolution of commonly found non-compliance issues. Working directly with facility and research staff to help them identify potential non-compliance before it happens provides one more layer of oversight. By explaining the "why" (issue), then providing the "what's" and "how's" (resolution), has demonstrated decreases the instances of non-compliance across the program. The PAM Program goes to great lengths to ensure that everyone is aware they can work with the PAM Team, the Veterinary services and support team, and the ACC, enabling them to make experimental changes while remaining in compliance. This is a critical component to creating a culture of trust while maintaining compliance and appropriate oversight. Overall, utilizing clear, concise communication, providing the necessary education, and facilitating resolution, are key to an effective and successful PAM program.

S4A4.4

Implementing a Fully Transparent Communication Policy of Animal Experimentation in a Swiss institution

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Abstract
The Swiss model, in which the population can amend the constitution through votes, has repeatedly given the issue of animal experimentation (AE) an important place in political and social debates. In 2022, the Swiss people voted for the fourth time to ban AE. With high societal concern for animal welfare, the scientific and pharmaceutical communities were worried. A fifth vote to ban AE is expected before 2030.

Until 2021, Lausanne's Federal Institute of Technology (EPFL), which has a well-known School of Life Sciences and medium-sized animal facilities, had limited communication on AE via its official communication channels. In 2022, the imminence of the vote served to catalyze the pre-existing desire for transparency, particularly within EPFL's animal facilities, and to put in place a progressive communication plan to inverse the situation. In addition to the rapid launch of a website packed with figures and images, and the prominence given to the issue in the school's press releases, numerous activities involving students, staff and the public have enabled EPFL to become one example of how transparency could be achieved.

We will show how this policy change was put in place, what the constraints were, and what tools enabled progress towards what is now almost total transparency. We will take a closer look at our regular facility visits, which enable a rich and immediate exchange with media and the public, inside and outside the school. We will explain why we believe they are essential for changing attitudes on this controversial subject.

S4A4.5

Understanding Regulatory Capture in Animal Research

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Abstract
The field of Laboratory Animal Science is a critical domain where ethical standards, scientific progress, and the welfare of animals are center stage. In this arena, regulation plays a pivotal role in balancing the needs of science while ensuring the responsible and humane treatment of animals. However, the effective regulation of animal welfare requires understanding the risk of regulatory capture (a phenomenon where regulations/regulator becomes overly influenced by one stakeholder). While public policy and regulatory

frameworks in democratic nations evolve with society's changing values and expectations, the regulatory agencies and governments that develop these frameworks risk becoming dominated by the industries they regulate rather than balancing these needs with public interests. Regulatory Capture is likely unintentional and can occur in various ways, including lobbying, financial processes, and crossover of personnel. Understanding the risk of regulatory capture in animal welfare regulations is crucial, as it can impact the effectiveness of the regulations, prioritize profits over welfare, lead to inadequate enforcement, and result in weak regulation, ultimately providing inadequate animal protection. Understanding the risk and the potential indicator of Regulatory Capture is essential in the evolution, development, and implementation of effective policies and regulations, protecting animals, and protecting the industry's legitimacy and democratic mandate to operate. This research investigates the signs and potential for Regulatory Capture in animal research to inform regulatory effectiveness and fit for purpose. Currently, his work is focused internationally on developing a methodology for evaluating animal research regulations in individual jurisdictions.

S4B1.1

Challenges and Emerging Trends in Reporting Actual Severity in Statistical Reports Under Directive 2010/63/EU

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Abstract
Directive 2010/63/EU requires the reporting of the actual severity experienced by each animal during the course of a procedure. Since 2015, "actual severity" has been reported in the EU statistical reports. Information on actual experienced severity plays an important role in pursuing the Three Rs by guiding decisions on future research needs and funding.

When this requirement was first introduced, only a few Member States had any experience in collecting data on or reporting actual experienced severity.

Although Annex VIII in the Directive provides some guidance on the prospective classification of severity, and additional guidance was agreed in 2013 which contained further examples, the assessment of severity throughout different stages of the project from planning to completion and final reporting was new to many Member States.

In the first few years of reporting, some common problems were clearly identified and rectified. However, some challenges remain. Moreover, new information on species capacity to experience pain, distress and suffering continues to emerge.

This presentation will review the trends and the need for better harmonisation of reporting of actual severity in recent years based on the annual reports published by the Commission, which collates data from each Member State.

Achieving consistency in severity reporting is crucial for ensuring animal welfare, fostering a level playing for the scientific community, and promoting transparency for the general public. Furthermore, it contributes to the evidence base for informing policy decisions and guiding research funding, ultimately driving more effective and responsible research practices.

S4B1.2

Lessons Learned from the FELASA Severity Workshops

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[6]*Radboudumc, Nijmegen, Netherlands*
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Abstract
Directive 2010/63/EU established the requirement for the classification of animal procedures based on their impact on welfare, promoting the need for more transparent and consistent reporting practices across Europe. To support this, the European Commission encouraged FELASA to organise Workshops on the Severity Framework, which have been conducted since 2016, mostly across Europe. These workshops serve as a platform for active input from researchers, technicians, and other stakeholders, aiming to improve understanding and implementation of the severity classification system.

Through these workshops, several key lessons have emerged. One significant finding is that many researchers and technicians remain insufficiently familiarised with the severity classification system, hindering accurate reporting and compliance with ethical standards. Additionally, it was observed that different EU countries apply varying criteria for classifying and reporting severity, resulting in inconsistencies and challenges in harmonising practices across the region.

Despite these challenges, there has been notable progress since the workshops began. Participants have gained a clearer understanding of the severity framework, and improvements in classification practices are evident. However, true harmonisation across Europe is still a distant goal. The divergence in national approaches underscores the need for continued education, collaboration, and the development of more unified guidelines.

This presentation will reflect on the key lessons learned from the FELASA Workshops, highlighting both the improvements made and the ongoing work required to achieve consistent and effective severity classification across Europe.

S4B1.3

How the Subject of Accurate Reporting of Actual Severity Has Been Addressed in France

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Abstract
In France, the collection of statistical data on the use of animals for scientific purposes is based on a rigorous framework that complies with European requirements. Beyond the reduction efforts undertaken by French user establishments, a key aspect of these declarations is the assessment of the actual severity degree of animal use in procedures, an obligation stemming from the European Directive 2010/63/EU. Until 2022, declarations of animal uses with a severe classification exceeded the European average.

In response to this finding, France focused its efforts on both prospective and actual assessments of the severity degree. Significant work was undertaken to train users in accurately reporting the actual severity level. These training efforts were formalized through webinars attended by several hundred users. During these sessions, exchanges with the scientific community revealed two key points: (1) an overestimation of the actual severity of experimental procedures, and (2) incorrect distinctions between prospective and actual severity levels, which could explain the high number of severe declarations.

In parallel, a new working group on severity assessment was initiated by the French 3R Center (FC3R), supported by national committees and the Ministry of Higher Education and Research. These various initiatives led to a significant decrease in the number of severe severity declarations in 2023, aligning with the European average.

S4B1.4

The Development of German Animal Numbers According to Prospective and Actual Severity

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Abstract
In Germany, the number of animals used in procedures is declining year on year. This applies in particular to experiments that were retrospectively classified as "severe", according to the pain, suffering, distress or lasting harm experienced by the animals. In a European comparison, only few experiments involving severe procedures take place in Germany. This could indicate that the actual severity is scored differently than in other EU member states. On the other hand, comparatively few projects involving severe procedures are authorised in Germany, according to national statistics. During project application, the prospective severity must be specified as part of the project application. This categorisation is carried out in accordance with the requirements of Directive 2010/63/EU and is subject to strict regulatory control. To this end, individual cases will be discussed to demonstrate how the prospective severity of procedures is determined during the authorisation process in Germany. In a second step, the methods used to estimate actual severity will be presented and this estimate will be systematically compared to the prospective severity. Further, if there are differences to other EU member states, these will be discussed. Finally, the long-term developments in the figures will be outlined.

S4C1.1

Achieving Results through Effective Change Management in Animal Care Practices

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Abstract
Achieving widespread adoption of Low Stress Handling (LSH) techniques in laboratory animal care requires more than technical training—it demands a robust change management strategy. Resistance to change, whether from individuals or institutions, remains a significant barrier to implementing progressive handling techniques, even when their benefits are well-documented. Using practical, research-driven approaches to change management, Charles River Laboratories has successfully implemented LSH in various housing strategies from isolators to ventilated racks at our sites globally. This presentation will explore Charles River's experience implementing LSH; how we gained buy-in, worked through logistics of this change, addressed multiple challenges through education and training, and measured success to drive sustainable implementation.

The presentation will focus on three core pillars of effective change management: 1) examine the importance of understanding the perspectives and motivations of key stakeholders, from animal care personnel to leadership teams, and provide strategies for aligning their goals with LSH objectives; 2) discuss methods to build communities of practice through social learning, empowering individuals to share successes, address challenges, and collectively develop innovative solutions; 3) highlight how digital tools—such as training platforms and real-time feedback systems—can enhance knowledge transfer and maintain accountability.

Real-world case studies will illustrate how these strategies have been successfully applied to assure LSH implementation across a global organization. Whether leading efforts within a single facility or across multiple organizations, attendees will leave with actionable insights and tools to navigate organizational resistance, foster buy-in, and sustain momentum to achieve transformational impact in rodent handling practices.

S4C1.2

Harmonising the Role of Designated Veterinarians in EU: Recommendations from a Joint FELASA-ECLAM-ESLAV WG

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Abstract
Article 25 of Directive 2010/63/EU requires Member States to "*ensure that each breeder, supplier, and user has a designated veterinarian (DV) with expertise in laboratory animal medicine, charged with advisory duties in relation to the well-being and treatment of the animals*". However, the implementation of Article 25 lacks harmonisation, with the DV's role and authority varying significantly due to national legislation and local policies, leading to significant variability between establishments and countries within the EU.

In response to this variability, FELASA, ECLAM, and ESLAV established a joint working group (WG) in 2021 to assess the current fulfilment of DV roles and similar positions globally. The WG aimed to develop recommendations to harmonise the DV role across Europe and support DVs in their duties.

This presentation will outline the WG's recommendations, which emphasise the recognition of the DV's authority, the necessity for professional expertise and specialist training, and the provision of adequate time and resources for DVs to perform their key tasks effectively. These measures are crucial for ensuring the welfare of animals under their care, particularly when DVs have additional responsibilities.

S4C1.3

The Norecopa Website - A Guided Tour of Global 3R Resources

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Abstract
The Norecopa website (norecopa.no) aims to be the leading one-stop-shop for global information about 3R resources, both in terms of content and frequency of updating. This presentation will give an overview of the website, much of which may be unknown to those who have not had time to explore it. Resources include:

- Extensive guidance on planning animal studies, including the PREPARE checklist in 36 languages (which may be used to construct a Study Plan) and links to over 400 guidelines
- A database of 1,800 textbooks and other literature within LAS and related topics
- A database of 3,400 alternatives or supplements to the use of animals in education and training, for use in schools, universities and animal facilities
- The website of the International Culture of Care Network
- An interactive map of 3R centres and LAS associations
- A newsfeed with the latest headlines from English and Scandinavian-language media covering the use of animals in research and testing
- An International Webinars & Meetings Calendar, continuously updated
- Links to events that have been recorded, sorted by the topics on the PREPARE checklist
- A searchable archive of the 125 newsletters issued by Norecopa
- Lists of course providers, funders and organisations that award 3R Prizes
- A Refinement Wiki, to which lab animal personnel can make their own contributions or submit material for uploading by Norecopa
- Pages with species-specific guidance
- Sections on key topics such as anaesthesia & analgesia, environmental enrichment, health monitoring, humane endpoints, humane killing, journals, legislation, and severity classification

S4C1.4

Enhancing Breeding Does Welfare in Research Facilities Through Innovative Housing

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Abstract
Breeding does in farms and research facilities are traditionally housed individually to manage breeding, prevent aggression, and reduce kit mortality. However, such housing systems fail to address the welfare needs of rabbits, including space for natural behaviors, enrichment and social interaction. Building on European efforts to refine group housing in farmed rabbits, we conceived, large, three-floor pens to specifically address welfare challenges associated with breeding does.

The use of vertical space overcomes floor space restrictions, resulting in wider usable surface area and increased rabbit housing capacity. The complex structure with straw/wood chip bedding, three levels, hiding spots, and litter boxes, allows does to express species-specific behaviors such as hopping, digging, hiding and social interaction. Recognizing the challenges of group housing, such as aggression and competition for resources, our design incorporates microchip-controlled doors (SureFlap, SurePetcare), ensuring each doe has exclusive access to her nest. Pens can be divided into two sections if aggression occurs. Nests have elevated space and internal feeding area for increased privacy. Kits will exit the nest only at the right age, when they will be microchipped. Additionally, connected feeding and watering systems (SureConnect, SurePetcare) enable individualized monitoring, ensuring optimal nutrition and care through detailed tracking of feeding and water intake, while facilitating the early detection of health issues.

These customized pens maximize housing density, enhance both physical and psychological well-being of the does, breeding performance and support the principles of Refinement and "One Welfare.", aligning with evolving societal expectations for animal care.

S4C1.5

Aging a Colony of Mice—Implications for Welfare Monitoring and Experimentation

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Abstract
Our understanding of laboratory animal behaviour and the implications of husbandry activities on their wellbeing remains incomplete. Home Cage Monitoring (HCM) provides valuable insights into mouse activity within the animal's own environment and can shed light on acclimatisation periods and responses to husbandry activities such as cage changing. The aim of this study was to monitor and explore changes in the activity and rest disturbance (RDI) patterns of an aging colony of male and female C57/BL6 mice. Mice were housed in the Digital Ventilated Cage® system, for up to 18 months of age. Habituation, aging and cage change assessments were conducted using linear mixed models, while cage separation and stereotypic behaviour investigations were conducted by visual inspection of the data. As they aged, the overall activity significantly decreased from 5 months until 14 months of age, after which it increased back toward baseline levels. Cages with mice flagged for potential stereotypy displayed sustained activity spikes in the light and dark phases. Cage changing led to an increase in the light phase activity and RDI compared to the previous day, with no observed difference in the dark phase post-cage change. This effect remained consistent as the animals aged. This study explored changes in the activity patterns of an aging colony of male and female C57/BL6 mice. We identified distinct aging phases concerning activity and RDI differences. In conclusion, the adoption of HCM systems could be considered for long-term animal housing from both a welfare and behavioural perspective.

S4C1.6

Stochastic Planning of Cohort- and Colony-Based Breedings

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Abstract
Breeding results are influenced by complex combinatorics and can be modeled by stochastic processes, requiring careful selection of various parameters, including the animals bred, maintained, and euthanized for each generation. Given the stochastic nature of these breeding processes, planning should be backed by specialized algorithms. We have developed a cohort-based breeding planner incorporating Mendelian genetics, fertility, and litter size, which is already available on our website (www.ltk.uzh.ch). We have now complemented this planner with an additional tool that facilitates stochastic planning for colony-based breeding. This new tool depends on the colony index (productivity) of specific animal strains or lines. It enables the calculation of the necessary number of breeders, taking into account the required confidence, colony index, shelf life, and weekly demand. This calculator is accessible via a user-friendly website. The impact of optimal breeding planning is considerable; it has the potential to reduce the number of surplus animals generated during complex breeding operations. Our calculator will make the planning of colony-based breedings more precise, helping to optimize the number of animals involved.

S4C2.1

Strategies to Prevent Single Housing of Male Mice: Chemical Sterilisation of Female Cage Mates

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Abstract
Individual housing of male laboratory mice, for a certain period of time, within experimental animal research, either intentionally or unintentionally, or as part of the breeding design for scientific purposes is common practice within animal facilities.

For animal welfare reasons, strategies within our local animal research institutes were evaluated to reduce the incidence and duration of single housing of male mice in order to reduce the associated level of discomfort.

This presentation describes the collaboration between research groups, animal facilities and the animal welfare body on this topic and the various (un)successful attempts and strategies adopted over time to reduce the discomfort associated with individually housing of mice.

One safe, reliable and refined approach to solve the situation of single housed male mice will be discussed in depth. This involves using chemical sterilization of a female cagemate with degarelix, a GnRH antagonist. This strategy is easy to apply, unwanted offspring is prevented, aggression when using a male cagemate is absent and the incidence and duration of single housed laboratory mice is reduced.

S4C2.2

Mice Just Want to Have Fun: The Impact of Toys on Mouse Welfare

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Abstract
Enrichment is not only important for improving the welfare of laboratory mice, allowing them to fulfil behavioural needs, but also a legal requirement. While there are many environmental enrichment options commercially available, most fall into one of the following categories: shelters, nesting/chewing material or climbing devices. Standard mouse housing, even with enrichment is far from the complexity of the natural environment and can lead to stress related behaviours such as inactivity, stereotypic behaviours and aggression.

The goal of this study was to design and produce a new type of enrichment to activate the animals, with a variety of difficulty levels, that would be more cognitively challenging, relatively easy to implement on a large scale and not occupy to much of the limited cage space. We therefore designed and 3D-printed a series of objects that would stimulate new behaviours, functioning more like toys than traditional enrichment. The mice were filmed in their home cages to evaluate the quality and quantity of interactions with the new objects and the overall effect on the animals' behaviour was analysed.

Results show that the mice interacted with all objects, with variations in total time and intensity of interactions between objects. Novel behaviours, not normally seen in the standard cage environment were observed and the results from an open field test showed an increase in exploration, indicating decreased anxiety levels and confirming that the enrichment has a positive effect on animal welfare.

S4C2.3

Good Nestbuilding – More to It than Providing Appropriate Nesting Material?

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Abstract
Nestbuilding behaviour and nest quality are well known parameters to assess animal welfare in rodents. At our University of Aberdeen facility, we sought to investigate this in more detail by comparing nestbuilding behaviour in different genetic altered strains and across our breeding and experimental units.

Baseline data were established using different nesting material (shredded paper, sizzle nest and cocoons). Subsequently, nest quality was compared in two different cage types followed by comparison of different age groups, and animal number per cage for different genetic altered mouse lines and the in house C57Bl6 colony.

Where the nest building score was below 3 nestlets were provided to establish if nest building behaviour could be improved.

In addition, to establish if behaviour was impacted when animals were under procedure, nest building was compared across our breeding and experimental units.

Our findings revealed the nest building score was higher in mice housed in shoe rather than in stock cages apart from one transgenic line. This score decreased when more than 4 mice were kept in one cage. However, nest building scores were similar for males and females. Finally, adolescent mice exhibited comparable scores to breeding pairs however, nest quality reduced by adult hood.

These data suggest nest building behaviour, in addition to nesting material provision, is affected by animal number per cage, cage design and genetic alteration but also the need of keeping warm and safe.

S4C2.4

GV-SOLAS Specialist Information: Breeding Planning for Laboratory Mice

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[5]University of Zurich/Institute of Laboratory Animal Science, Zurich, Switzerland

Abstract
Efficient and effective breeding planning is pivotal in biomedical research to reduce the number of animals required for generating experimental cohorts and maintaining rodent colonies. This presentation discusses the significance of meticulous breeding approaches and strategies employed to achieve the necessary animal numbers while minimizing surplus.

The Committee for Genetics and Laboratory Animal Breeding of the GV-SOLAS has developed an in-depth guide, catering to skilled professionals, facilitating the design of tailored breeding programs to meet specific research needs. The primary objective is to optimize breeding outcomes and minimize the number of experimental mice and surplus animals.

To accurately plan breeding procedures, a comprehensive understanding of genetic characteristics and other pertinent parameters influencing breeding outcomes is pivotal. By utilizing standardized breeding methods, various types of breeding can be categorized, allowing for the precise calculation of breeding animals and expected offspring within a well-defined confidence framework.

In this presentation, we outline the process of breeding planning, emphasizing the importance of addressing potential challenges while striving to generate suitable experimental cohorts. Additionally, we delve into the limitations of achieving cohort breeding without generating surplus animals. By raising awareness about the intricacies involved in breeding planning, we aim to encourage researchers in the biomedical field to engage in responsible and efficient animal utilization.

S4C2.5

AI as a Tool for the Refinement of Pain Recognition in Rodents

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Abstract
Since pain and stress in laboratory animals can impair the accuracy, reliability, and relevance of the research data collected from animals (1), and since pain affects data from the entire organism (blood pressure, heart rate, respiratory activity, body weight, immune system, wound healing, etc. (2)), study results can be distorted, leading to inadequate results and unguaranteed reproducibility and transferability of the findings.

We developed an interface-independent application for the automated recording and assessment of pain in laboratory animals. This is based on the so-called Grimace Scale using AI. The Grimace Scale is a validated methodology that captures defined changes in facial expression (e.g., eye shape and opening, position of ears and whiskers, and facial contour (3)) for meaningful and consistent pain recognition. However, since the method is mostly implemented manually today and, due to this increased effort, is not part of the generally mandatory procedures, we aimed to transform individual observation into a consistent, continuous, and objectively verifiable method and to explore a relevant market in the field of laboratory software. By capturing data in real-time and increasing the speed, precision, and effectiveness of the methodology, this can contribute to the standardization of pain recognition in the future. Our user-friendly system can simultaneously enhance effectiveness in laboratories.

S4C2.6

Implementation of Digital Animal Welfare and Humane Endpoints through Home-cage Monitoring Solutions

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Abstract
The introduction of digital home-cage monitoring solutions is revolutionizing laboratory animal research, particularly for rodents, through continuous and non-invasive welfare monitoring. These advanced systems refine humane endpoints and automate numerous vivarium processes. By enabling researchers to gather comprehensive datasets in real-time, these technologies optimize study designs, enhance data quality, and reduce animal use — aligning with ethical research practices focused on the 3Rs. In this talk, we will examine the practical applications and outcomes of digital home-cage monitoring implemented by Novartis globally. Our presentation will demonstrate significant improvements in several areas: enhanced animal welfare, increased research process efficiency, and improved data reliability and reproducibility. These case studies will highlight how these digital monitoring solutions drive these advancements, ensuring that animal welfare is enhanced without compromising scientific integrity. Furthermore, the adoption of these innovative systems is prompting a reevaluation of traditional laboratory animal practices. Experts are now empowered to integrate continuous monitoring into their daily routines, fostering better care and management. We will explore practical strategies to adopt these technologies, showcasing their advantages such as cost savings, improved data accuracy, enriched animal welfare, and enhanced scientific rigor. This paradigm shift not only facilitates groundbreaking research but also upholds the highest standards of animal treatment, setting a new benchmark for future practices.

S4D1.1

Psychological Stress and Strain in Laboratory Animal Professionals

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Abstract
The occurrence of psychological strain in laboratory animal professionals (LAP) has been demonstrated in several studies[1–3]. However, published data do not allow reliable statements on which specific stressors and moderating factors are relevant for the development of strain in LAP.

Therefore, we aimed to assess potential stressors to which LAP in German-speaking countries are exposed and to analyse their impact on psychological strain.

In an online survey, participants' mental state was assessed using the General Health Questionnaire (GHQ-12). Potential stressors were assessed using questions from the Copenhagen Psycosocial Questionnaire (COPSOQ) for common general stressors, as well as tailored questions on potential job-specific stressors.

The survey was conducted in the summer of 2023 and included a sample of 1195 LAP with anonymous participation.

Hierarchical regression analysis was used to assess the impact of demographic factors, as well as general and domain-specific stressors, on participants' psychological state. Results indicate that both general and domain-specific stressors are relevant in LAP and that strain is also influenced by demographic factors.

The data from this study can contribute to the development of general protective measures against psychological hazards in the workplace of LAP. They may also help institutions involved in animal experimentation to set priorities in the risk assessment of mental stress.

Taken together, the results can help to reduce negative effects on LAP, the animals and the research results, and can help to promote a culture of care.

S4D1.2

Personal Impact of Animal Use: (Tangential) Insights from an interview study with UK researchers/students

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[2]i3s - Institute for Research and Innovation in Health, Porto, Portugal

Abstract
This presentation reports insights from a qualitative study focused on understanding sociocultural factors around the uptake and acceptance of non-animal methods in UK academic institutions and decision-making around model choice. Despite not being part of the interview agenda, the personal impact of animal use came up in multiple interviews. The presentation will contribute insights and reflections to stimulate discussion. Important questions include: What happens when researchers using animals feel doubt or uncertainty around the value or relevance of their work? If the harms do not seem to outweigh the benefits in practice? Do researchers have others with whom they can share their doubts?

The talk will be presented as a video recording.

S4D1.3

I Am Not Sure I Want to Do It: Emotional Perspective on Animal Experiment

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Abstract
In Italy students and early-year researchers are requested by law to follow training courses to familiarise with procedures carried out on research animals. During these courses, the attendants could experience emotional distress when asked to perform invasive procedures and/or to sacrifice experimental subjects. A qualitative analysis of interviews with students and early researchers of the Center for Behavioural Science and Mental Health at the Istituto Superiore di Sanità in Rome will be precsented. The interviews focused on the potential emotional difficulties encountered, and the way to solve such difficulties. The extent of invasive procedures during training, as well as during early experiences in a research project, related to injections, and gavages. The self-trust in carrying out such procedures in a professional manner was the main factor tu overcome the unpleasentness of the procedures. The sacrifice of animals resulted to be the most critical aspect. The majority of respondants can do it when they manage to put aside the emotional aspect of killing a living being, and put forward the need for science. The ones who cannot follow this mental process will not sacrifice animals. Related to this, it appeared that one-on-one relationship with a tutor or supervisor was by far preferred to the participation in organised courses. The crucial factors in addressing by staff members emotional distress in students were: give time and respect; neve force anybody to do anything they dont feel like doing; understanding emotional diversity; empathy and dissipation of fears; re-locate tasks within a project.

S4D1.4

Who Should Learn to Do Experiments with Animals and How?

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Abstract
The use of animals in research is ethically problematic, in particular through the harm that is caused to laboratory animals. Whereas much has been invested in addressing the ethical issues on a collective level, much less attention has been given to the moral quandary of animal experimentation in the context of individual ethics. Learning to do experiments with animals continues largely to be the "rite of passage" for researchers-to-be that social scientists Birke, Arluke and Michael described in 2007. As teachers and mentors, we increasingly meet early career researchers in training who find themselves in a real quandary over using animals in their research, even though they have committed to a graduate research project with animals and signed up for a course in order to gain the required competence. We will argue that it is time to discuss how best to support these future professionals in a more constructive way than what we believe is presently the case. We will share our experiences of changing the way we approach the question of animal experimentation and moral distress when training inexperienced early career researchers.

S4E1.1

Implementation of AI Based Monitoring in Rodent Preclinical Studies

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Abstract
Algorithms and artificial intelligence (AI) are used more widely in biomedical research or routine patient care, and their benefits are well identified to optimize study monitoring and regulatory submissions. But they can also play a part in preclinical in vivo studies by helping in discriminating groups and identifying treatment-induced or adverse effects at the early stages.

The development of digital cages enabling measures of locomotor activity, followed by home cage video-monitoring solutions, are progressively modifying preclinical rodent study designs. Those systems have already demonstrated their potential to enrich study outcomes and detect both clinical signs and ethical endpoints in rodent studies better than routine daily checks, and the development of new AI algorithms will further fulfill the expectations of trained and creative scientists.

Different use cases will be presented to demonstrate the benefit for scientists and veterinarians, also generating growing

interest from authorities to increase animal welfare and scientific robustness.

S4E1.2

An Innovative Acoustic Approach Put to the Test

J.-B. Prins[1], M. Raspa[2], M. Garzola[3], L. Rignanese[3] and G. Rosati[3]
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Abstract
Accurate and timely determination of pup birth in biomedical research and breeding settings remains an ongoing challenge, with often delayed observation of pups born. Historically, methods based on visual cage inspection, controlled mating and of prepartum symptomatology are inadequate to meet precise research timelines. An innovative approach with automated detection methods was deemed essential to fill the gap. Microphones were introduced into standard IVC Cages (Tecniplast EM500). The acoustic signals were analysed real time through AI technology allowing the identification of specific vocal patterns in the audible range (up to 20KHz). The potential of this technology was tested in a breeding situation as a means of birth detection. We focused on the detection of low-frequency wriggling calls emitted by mouse pups starting from the mating date onward. The study was performed at three centres: CNR-IBBC in Rome, The Francis Crick Institute in London and The Leiden University Medical Centre. To each cage, a pregnant C57BL/6 female with or without male was continuously monitored along the time of pregnancy and after the parturition until the weaning of the pups. Results will be presented from different breeding configurations.

S4E1.3

Personalised Gene Therapy Combined with Home-Cage Monitoring in Mice

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Abstract
In recent years, we have witnessed a transformative revolution in gene-editing technologies, particularly with the development of clustered regularly interspaced palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins. Leveraging these advancements, we have created an innovative system utilizing a modified catalytically inactive Cas (dCas12f). This system is designed as a programmable and tissue-specific epigenome editing tool.

The primary goal of our invention is to establish a control system capable of precisely and timely regulating the expression of specific mammalian genes.

To optimize the effectiveness of our epigenetic interventions, we use a daily home cage monitoring system for mice. This advanced monitoring technology allows us to continuously and accurately assess changes in circadian parameters in real-time. By integrating this system, we can meticulously monitor the effects of our treatments and adapt them to develop a chronotherapy approach. This strategy ensures that the administration of treatment is perfectly aligned with the natural rhythms of the subjects, thereby maximizing therapeutic outcomes.

S4E1.4

AI to the Rescue: How AI Tools Can Help NACWOs, NTCOs, and NIOs Roles

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Abstract
Named Animal Care and Welfare Officers (NACWOs), Named Training and Competency Officers (NTCOs), and Named Information Officers (NIOs) play a critical role in ensuring compliance with animal welfare regulations, fostering a culture of care, and developing staff competencies in laboratory animal science.

However, increasing administrative workload, the need to develop engaging training materials, and deliver effective communication can be time-consuming. AI tools, such as Microsoft's Co-Pilot, are emerging as potential allies in addressing some these challenges.

At AstraZeneca, the use of Copilot, an AI-driven assistant embedded within Microsoft applications, was tested with the goal of streamlining administrative tasks within the Animal Sciences and Technologies Department. Examples of successful implementation include: (1) generating automatically compliance meeting minutes, (2) facilitate reviewing and updating training materials, (3) creating presentations, quizzes, checklists or frequently asked questions documents from internal policies and SOPs and (4) summarizing animal welfare or accreditation regulations for members of the *in vivo* research community.

Although time saved by using these AI tools can be significant, it is crucial to implement them responsibly to prevent unintended consequences. It is essential to promote adequate training and understanding of these tools, retain personal responsibility for their ethical use and critically oversee the all the material generated.

A robust and appropriate use and oversight of AI-tools ensures that NACWOs, NTCOs, and NIOs can concentrate on what matters most: advancing animal welfare and fostering a culture of care in laboratory animal facilities.

S4E2.1

Pyrogenicity Paradigm Shift: Moving from Rabbits to Human-relevant Research

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Abstract
Efforts to develop ethical, human-centric methods for assessing health risks have grown, fostering opportunities to move away from animal testing. Despite this, nearly 1 million animals were used for regulatory testing in Europe from 2018–2022, with over half for product quality control (QC). In batch safety and potency testing, non-animal alternatives offer potential, but adoption faces historic, scientific, and regulatory barriers.

Pyrogenicity testing exemplifies progress in overcoming these challenges. Historically this test was reliant on the Rabbit Pyrogen Test (RPT), but its ethical and scientific limitations spurred the development of in vitro alternatives like the Monocyte Activation Test (MAT). MAT is now validated and accepted in Europe, United States, Russia, and India. However, so far, only the European Pharmacopeia (Ph. Eur.) has phased out RPT, reflecting MAT's integration as a compendial method. On the other hand, in the US, RPT is still considered the gold standard, and MAT is not considered compendial to RPT. This illustrates that MAT adoption remains inconsistent globally. Regulatory inertia and slow progress in phasing out RPT are highlighted as disparities.

This presentation analyzes the phase-in of MAT and RPT phase-out by examining scientific, historical, and regulatory factors. It highlights Europe's slow but steady shift, nearly 40 years in the making, and the US lag due to regulatory inertia. We find that accelerating transitions requires parallel method phase-ins and phase-outs, proactive legislation prioritizing non-animal methods, and incentivized regulatory frameworks to make animal testing a true 'last resort'.

S4E2.2

Tracing Replacement of the LD50 Bioassay for Botulinum Neurotoxin (Botox)

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Abstract
Botulinum neurotoxin (BoNT), more commonly referred to as "Botox", is widely used in medical and aesthetic applications, with over 11 million aesthetic treatments performed in 2019 in Europe alone. As a biological product, BoNT requires rigorous batch potency testing to ensure safety and is currently reliant on the ethically and scientifically contentious mouse lethality assay (MLA). Despite the reproducibility crisis and translatability issues of this LD50 test, its use persists due to regulatory inertia and systemic barriers.

Recent advancements in new approach methodologies (NAMs), particularly cell-based assays, offer promising alternatives for final product testing. However, adoption is hindered by the lack of universal standards, product-specific variability, and the necessity to validate NAMs against MLA data. Furthermore, regulatory loopholes exempting BoNT from animal testing bans exacerbate these challenges, even when its primary use is aesthetic/cosmetic. This presentation draws on Geels' multilevel perspective framework to explore the scientific, regulatory, and societal factors affecting the transition to NAMs. It highlights:

1. Limitations of MLA and progress in NAM development,
2. Stakeholder roles in sustaining or challenging MLA reliance,
3. Policy barriers and regulatory steps for phasing out MLA in the European Pharmacopeia, and
4. The societal influences driving demand for aesthetic BoNT use.

By synthesizing findings from literature reviews and stakeholder interviews, this presentation provides actionable insights into overcoming barriers to implementing ethical and scientifically robust alternatives in regulatory testing, which aligns with Europe's commitment to advancing humane animal research practices and ultimate Replacement.

S4E2.3

Unavoidable Breeding Surpluses: Is Killing and Feeding of Surplus Laboratory Animals a Criminal Offence?

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Abstract
Due to Mendel's rules, the breeding of genetically modified animal models inevitably results in a large number of animals that could not be used in research projects due to their unsuitable genotype. In Germany 2.44 million animals were used in animal experiments in 2022, but also 1.77 million 'unused, killed animals' were reported (97% mice). The ratio is similar in the 27 EU member states.

Adequate breeding planning and optimisation of the experimental conditions can reduce this number of unused animals, but cannot completely avoid it. The breeding surpluses can be utilised for other purposes only to a small extent. This creates a considerable ethical problem. In Germany, there is also a legal problem because the killing of animals without good reason is prohibited by law.

Another option would be to feed these animals to carnivores in human care, for example in zoos. However, this is only possible for wild-type animals, but not for genetically modified animals, which are considered genetically modified feed within the meaning of EU Regulation 1829/2003. For each of the large number of mouse lines, an individual authorisation as animal feed is required, which is so time-consuming and costly that it is impossible to supply these animals for animal feed.

We present a solution for this ethical problem: the EU Commission would have to exclude surplus research animals from the scope of Regulation No. 1829/2003 by applying Article 15(2). This would allow the use of surplus genetically modified laboratory animals as animal feed throughout the EU.

S4E2.4

Performance Evaluation Program for Diagnostic Laboratories: Key Instrument for Safe Global Exchange of Rodents

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Abstract
The International Council for Laboratory Animal Science (ICLAS), with the support of several diagnostic laboratories around the world, has developed a self-evaluation program for diagnostic laboratories to assess their diagnostic performance: the Performance Evaluation Program (PEP).

The program consists of the preparation of well-characterised sera and microbiology specimens and their distribution to participating laboratories.

Since its creation in 2006, 45 laboratories from around the world have participated, and nearly 400 different specimens, prepared by six diagnostic laboratories worldwide (ICLAS Network laboratories, located in Europe, Asia, America and Australia) have been distributed.

PEP is a self-evaluation program, and although laboratories are not obliged to report back, they are asked to provide anonymous voluntary feedback on their results to share knowledge and help improve the program.

The program focuses on those specimens that are currently relevant for the health monitoring and safe exchange of rodents as outlined by international published guidelines, i.e. FELASA.

Over the last 18 years, the PEP program has distributed positive sera and microbiological samples for all of the agents recommended by FELASA guidelines to check when performing health monitoring for mice and rats. The detailed library of samples will be presented in detail at the congress.

Each year, new diagnostic laboratories join the program - demonstrating PEP's usefulness as a tool for quality assurance to check and improve diagnostic performance and therewith to enhance laboratory animal quality.

S4E2.5

No More Canaries in Coal Mines: A Systematic Review of Environmental Health Monitoring

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Abstract
Although traditionally rodent health surveillance used live Soiled Bedding Sentinels (SBS), increasing evidence is demonstrating that Environmental Health Monitoring (EHM) is more effective while also promoting the 3Rs and reducing costs. Still, as demonstrated in benchmarking surveys, some institutions have still not switched, sometimes citing lack of scientific evidence for EHM. Therefore, to fully establish the evidence base for EHM, our team performed a systematic literature review to identify, summarize, and evaluate the efficacy of EHM as compared to SBS.

After systematically searching and evaluating articles from established databases published prior to October 15, 2023, there were 42 peer-reviewed publications included in the analysis. Although their design varied, they included evaluations of exhaust dust testing (n = 27), sentinel-free soiled bedding (n = 8), and direct colony sampling (n = 24). Analysis showed that all types of EHM were superior to soiled bedding sentinels at detecting pathogens, regardless of pathogen type.

Therefore, there is a strong evidence base for replacing SBS with EHM. Furthermore, in conjunction with this project, the 3Rs Collaborative's EHM initiative has created an extensive resource hub on this topic including lists of institutions that have switched, downloadable SOPs and slide decks, advice on how to switch, a mentorship program, cost analysis, and more. This presentation will therefore provide the evidence for and resources to replace SBS with EHM which can be used as a case study for how new technologies can be integrated in animal resources.

S4E2.6

Next Research – Closing Animal Welfare Gaps

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Abstract
New footprints on old paths: One of the current research focuses at the Center for Preclinical Research is "Next Research". This describes a modern approach in science in which we consciously focus on our responsibility towards nature, especially in the context of animal welfare. The holistic old approach "everything is connected" is the basis for this. To achieve this, we combine scientific methods with entrepreneurial practical approaches and an ethical framework.

Guiding principle: "Next Research" projects should have a practical and beneficial relevance for animals as well as an ethical and possible legal impact.

For example: It's time for analgesia for chicken embryos

In the EU, procedures on chicken embryos are not considered as animal experiments and the use of chicken embryos is even accepted as an alternative method to animal testing.

We will describe how we designed and conducted studies to investigate nociception in chicken embryos and how the results led to an amendment of the German Animal Welfare Act.

We demonstrated that chicken embryos show physiological electrical brain activity from day 13 after hatching. Consequently, the ability to perceive aversive sensory experiences as pain can no longer be ruled out from this point on. Furthermore, from day 15 of incubation, chicken embryos respond to a noxious stimulus with changes in the cardiovascular system and behavior, indicating the ability to nociception. In terms of the refinement of animal experiments, we therefore consider it necessary to use effective analgesia in chicken embryos during potentially painful procedures.

S4E3.1

Expansion of Assessment Criteria for Function B persons as Defined in the Directive

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Abstract

This ETPLAS Working Group created assessment criteria for evaluating each of the Education and Training (E&T) Framework guidance document Learning Outcomes for the specific Modules for those designing procedures and projects involving animals for scientific purposes (Function B persons).

The assessment criteria address knowledge and skills, as well as critical thinking and appropriate attitudes, which are expected to be acquired during E&T of persons designing procedures and projects. It is appreciated that the learners may have diverse prior experiences and expertise, therefore we created assessment criteria for two levels of understanding for each Learning Outcome, an ideal level of understanding and one that would be acceptable. Examples of these two assessment levels and the differences in the wording will be shared. Course providers and assessors may select the most appropriate level of attainment for each Learning Outcome pertaining to their local needs, as well as the format of written assessment (see[1]).

Additionally, expanded assessment criteria for the Learning Outcomes for persons carrying out procedures on animals (Function A) and for those taking care of animals (Function C) have been described in our previous publication[2]. Their wording permits the evaluation of the knowledge acquired by objectively assessable examination methods.

The creation of these assessment criteria and their use by course providers, Accrediting Bodies and other stakeholders will support the harmonization of assessments for Function A, B and C courses across the EU, enhance the quality assurance, and support free movement of scientists in Europe.

S4E3.2

Union Wide Continuing Professional Development (CPD) Framework for the Field of Laboratory Animal Science

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Abstract

Animals may only be used for scientific purposes when no alternatives exist, when any harm caused to them is minimized, and when the scientific output is of the highest quality. Ensuring this requires the competence of everyone involved, which is supported by legal requirements mandating appropriate education and training before engaging in the care and use of animals for scientific purposes.

Continuing Professional Development (CPD) is an ongoing process that enables individuals to maintain their current knowledge, skills, and competencies, as well as to acquire new ones. In the European Union, within the context of using animals for scientific purposes, Article 24(1)(c) of the EU Directive specifies that it is the responsibility of designated persons to ensure that staff are properly educated, competent, and continuously trained. As a result, CPD is a legal requirement for all those involved in the care and use of animals.

The ETPLAS working group has proposed a Union-wide framework for CPD in laboratory animal science (LAS). This framework is designed to be adaptable to countries that have already established CPD systems, as well as those developing them. Its goal is to harmonize CPD practices across Europe and beyond, thereby facilitating the mobility of individuals involved in animal care and experimentation.

The framework outlines methods for quantifying CPD activities based on their type and proposes a protocol of CPD activities for each individual, with the support of the designated person outlined in Article 24(1)(c) of the EU Directive.

S4E3.3

DOPS Templates: Supporting Harmonized Assessment of Practical Skills in Animal-Based Procedures

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Abstract
Assessing practical skills is essential to ensuring competence in laboratory animal science (LAS) and allowing individuals to work independently with animals without supervision. Direct Observation of Procedural Skills (DOPS) stands out as a validated and structured method for evaluating practical competencies using templates specifically designed to assess trainee's skills. These templates are aligned with the European Training Platform for Laboratory Animal Science (ETPLAS) framework and learning outcomes for specific modules and promote a consistent and harmonized approach to LAS education and training within and beyond Europe. DOPS templates provide transparent feedback and support targeted skill development, ensuring that personnel involved in animal procedures meet the high competence standards agreed upon by EU member states. These DOPS templates help ensure compliance with the legal requirements of Directive 2010/63/EU regarding personnel competence and advance the directive's objectives of harmonization and transparency across institutions. In summary, by ensuring high levels of practical competence in hands-on work with animals, DOPS promotes better animal welfare, enhances scientific quality, and can help reinforce public trust in animal-based research.

S4E3.4

The ETPLAS Training Platform and its New EU E-learning Modules

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Abstract
Alongside working to improve and harmonize education and training in laboratory animal science (LAS), ETPLAS is known for hosting on its website six free e-learning modules, some of which new to the original Education and Training (E&T) Framework.

In three years, around ten thousand users registered and completed +13.000 courses. The impact of these free courses, used both by course organizers as reference material, and by individual users for continuing professional development (CPD), is now expected to increase even further, as 17 new course modules were developed in 2024 under the EU-funded LAS-Learning project, and will be made freely available in 2025. These include core modules EU-2 and EU-9, "Ethics, Animal Welfare, and the 3Rs" (Levels 1 and 2, respectively), as well as function-specific modules for Function A, namely "Basic and Appropriate Biology" (EU-3.1), and "Recognition of Pain, Suffering, and Distress" (EU-5) for mice, rats, zebrafish, pigs, ruminants, and chickens. This new online educational offer will moreover include modules for specific roles, such as "Designated Veterinarian" (EU-24) and "Inspectors" (EU-26), as well as the new EU-27 module "Competence Assessors".

Developed by over 20 laboratory animal scientists and veterinarians, revised by a reflection group of +50 LAS professionals, and tested by +200 users, the new modules strive to provide high-quality e-learning resources that can integrate seamlessly into existing curricula, serve as stand-alone training tools, or be used for CPD by the scientific community. This talk will showcase these educational materials and explore how they could benefit trainers and learners alike.

S4E4.1

Anaesthesia and Analgesia for Cattle, Sheep, Goats and Pigs Used in Biomedical Research

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Abstract
This FELASA Working Group (WG) is currently finalising the guidelines for anaesthesia and analgesia for mammalian farm animals used in biomedical research. Wherever possible, evidence-based recommendations have been made for: 1) safe and effective anaesthesia and analgesia for farm animal species; 2) sedation, restraint and regional anaesthesia; 3) monitoring during anaesthesia; 4) management of anaesthetic complications; 5) pain assessment and management; 6) contextualised care within scientific methodology and its associated constraints. The existing literature on farm animal anaesthesia and analgesia focuses mainly on surgical husbandry procedures rather than complex experimental procedures. The WG has developed recommendations based on both personal experience in laboratory animal practice and selected available guidelines. The recommendations provide guidelines for the implementation of safe anaesthesia in farm animals in biomedical research.

The recommendations are made with consideration of legislative frameworks and the 3Rs. Ultimately, safe and acceptable protocols for anaesthesia and analgesia in farm animals require:

1) appropriate professionals with a high level of relevant competence for the target species; 2) anaesthesia and analgesic schemes that accommodate scientific outcomes whilst ensuring optimal animal welfare.

In this presentation, the WG summarises the final status of the recommendations developed and reports on the considerations for establishing and selecting anaesthesia and analgesia regimes for farm animals used in animal experiments.

S4E4.2

The Pig as a Model in Translational Surgery

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Abstract
Large mammals are required for surgical research since rodents do not allow the translation of the procedures to humans. The Pig has a particularly high translational value due to its similar overall anatomy and physiology.

It is therefore the most used species in the fields of cardiovascular and digestive surgery. In cardiovascular surgery, its contribution has been important for stents development, coronary bypass grafting improvement and heart valve xenotransplantation. In digestive surgery, the utility of pigs has been diverse, with the development of meshes for abdominal defects repair or improvement of surgical procedures aiming at compensate functional defects. A particular utilization has been made in liver regeneration and transplantation procedures and in metabolic surgery research for metabolic diseases interventional therapy.

In endocrine surgery, the use of the Pig has mainly consisted in the development of pancreas and islet transplantation for type 1 diabetes therapy. Osteoarticular and neurosurgery are fields where the use of the Pig is increasing.

The Pig has a particular present and future involvement for testing new surgical equipment or bioengineering solutions, developing new minimally invasive approaches and robotic surgery training.

Its major involvement for the future would undoubtedly be as an organ-supplier for human xenotransplantation. First trials have been achieved in humans but there is still a long way to demonstrate the long-term safety and efficacy. Using pigs for this purpose is also a challenge that would have to deal with societal expectations regarding the routine use of animals in biomedical research in respect with the 3Rs.

S4E4.3

Moving Towards a More Rigorous Understanding of Mouse Behavior and Physiology

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Abstract
Ongoing efforts to promote the 3Rs (Replacement, Reduction, and Refinement) in animal research underscore the need for innovative approaches that enhance scientific rigor while respecting animal welfare. Traditional tools like the rotarod and open field tests, while widely used to evaluate mouse behavior and physiology, introduce stress, disrupt natural circadian rhythms, are inherently qualitative, and offer limited insight into key physiological parameters such as sleep patterns. These limitations not only impact animal well-being but also introduce data variability, complicating the interpretation of results and hindering reproducibility.

To address these critical gaps, The Jackson Laboratory has developed Envision™, an AI-enabled cloud-based software platform that leverages extensively validated computer vision routines capable of continuously and quantitatively measuring mouse behavior and physiology in a non-invasive manner. Data analyzed by Envision is collected via the Allentown Discovery™ IVC, which integrates video-based monitoring in the home cage environment. This system delivers both off-the-shelf digital measures as well as enables users to develop their own digital measures, opening up near limitless potential to automatically track behavior and physiology in the home cage.

This talk will highlight the application of Envision to continuously track behaviors of mice across both the light and dark cycle, demonstrating statistically significant differences in both sleep patterns and neuroactivity that emphasize how Envision enables new insights into mouse phenotypes.

S4E4.4

Validation of the Wildling Mouse Model to Enhance Translational Research

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Abstract
The Wildling mouse model, characterized by a natural **microbiome**, offers an innovative approach to preclinical research by **more closely resembling human immune system dynamics** than traditional specified pathogen-free (SPF) mice. A **collaborative effort** involving nine research groups, the local lab animal facility, and Responsible PrecliniX investigates the external validity and applicability of this model across diverse contexts.

This project evaluates Wildling mice under varying conditions, including health studies with different diets, behavioral phenotyping, and disease models such as influenza, nosocomial lung infections, acute kidney injury (AKI), cystic fibrosis, inflammatory bowel disease, Alzheimer's disease, cancer (colon, liver, pancreas), and stroke. CCR5-targeting immunomodulatory therapies are also being tested in Wildling and SPF mice for influenza, AKI, and stroke. By systematically comparing the two models, the research aims to **identify scenarios where Wildling mice outperform, align with, or underperform relative to SPF mice**. Even when their performance is comparable or limited, the Wildling model's mature immune system may provide valuable insights, particularly for immunomodulatory therapies. A meta-research component supports this effort, offering a broader perspective on its translational potential.

Supported by harmonized protocols, quality assurance, and rigorous experimental designs, the team has successfully bred transgenic and wildtype Wildling mice at Charité[1]. Preliminary findings indicate that the model's immune traits may improve preclinical studies' translatability and external validity[2]. This research seeks to advance biomedical science by refining preclinical models to address complex disease mechanisms better.

S4E5.1

FELASA - Its Visions, Missions and Liaison

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Abstract
The Federation of European Laboratory Animal Science Associations (FELASA) is currently composed of 21 full member associations representing 28 countries, and an affiliated member organisation. Founded in 1978, FELASA's mission has ever since been to represent the common interest of its members, for the furtherance of Laboratory Animal Science (LAS) in Europe and beyond. Laboratory animal scientists work towards ensuring optimal conditions for the humane use of animals for research in an ethically responsible framework, with the 3Rs as a guiding star. FELASA is particularly active to promote good practice through the organisation of conferences and workshops, production of recommendations by expert working groups, accreditation of education and training programs, and through networking and collaboration with its international liaison bodies. This presentation will give an overview and explanation of how FELASA works, in order to provide scientists and other stakeholders involved with the use of animals in research and education, an understanding of how to interact with FELASA, to participate in its events and activities, and to follow FELASA's guidance and achievements. We will also highlight how FELASA adapts to nowadays challenges and proposes further developments. Further, FELASAs three international liaison bodies – AALAS, EARA, and ICLAS – will present their organisations and collaboration with FELASA, for the strengthening of Laboratory Animal Science at a global level

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S4E5.2

AALAS and FELASA Working Together

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Abstract
Since 2012, The Federation of European Laboratory Animal Science Associations (FELASA) and the American Association for Laboratory Animal Science (AALAS) have been collaborating to harmonize animal care and research recommendations for the Laboratory Animal Science (LAS) community. A Liaison Body comprised of individuals from each association was established to advance information exchange and to oversee the establishment of joint working groups. The working groups are populated with experts from both organizations. Each working group is charged with exploring a specific topic of interest and importance to the

LAS community and publishing recommendations for best practices. This session will provide a brief overview of AALAS and will outline the structure and relationship between FELASA and AALAS. Additionally, there will be a summation of the efforts of current and past working groups. The audience will learn more about the relationship between the two organizations and how they use joint working groups to promote best practices across the globe.

S4E5.4

The International Council for Laboratory Animal Science - ICLAS

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Abstract
ICLAS is an international scientific non-profit organization dedicated to advancing human and animal health by promoting the ethical care and use of laboratory animal science. Its members are located on all continents.

Founded in 1956 under the auspices of UNESCO, ICLAS has continuously expanded its reach and influence. The initial idea was to elevate the standards for using laboratory animals globally, and since then, ICLAS has grown into a thriving international organization.

ICLAS, with its diverse membership including national, scientific/union, institutional, associate, and affiliated members, represents a strong network with regional committees in Africa, America, Asia, Oceania, and Europe. ICLAS collaborates closely with FELASA and the EARA within the EU, reinforcing a sense of unity and shared goals.

ICLAS' goals are:
- promotion of ethical principles, scientific responsibilities and animal welfare in research and education
- collaborate within the global LAS community
- be a resource for the worldwide community of knowledge and best practice in LAS
- advocate for the advancement of LAS in developing countries and regions

In 2006, a joint initiative was set up between ICLAS and laboratories involved in the health monitoring and/or genetics of laboratory animals, including the two networks *Performance Evaluation Program for Diagnostic Laboratories and the Genetic Quality Monitoring Program*.

ICLAS has scholarships for veterinarians, and grants for "Training the Trainer in LAS Education in Europe" and the "Visiting Grant for Professional Development in LAS in Europe". The last two are for ICLAS EU members or member organizations.

S4F1.1

Introduction: Training Rodents – Principles and Challenges

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Abstract
Rats and mice used in research are often subjected to stressful procedures reducing the welfare of the animals. The EU directive 2010/63/EU prescribes that "Establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project" and additionally training the animals can affect the severity category of an experiment.

Rodents such as mice and rats are seldom trained as such. Often, researchers will state that the animals are habituated to a certain procedure; however, this can in some cases be questioned. Training the animals using operant or classical conditioning with the purpose of improving the welfare during handling, husbandry and procedures are seldom done. In this talk, the principles of habituation, classical and operant conditioning will be presented including videos showing different ways of training mice and rats. The benefits of animal training will be discussed

There are several challenges, when considering training rodents for handling and experimental procedures. These challenges may relate both to available resources and to the animals. Never-the-less the benefits of animals training should be considered and balanced case-by-case. Whenever possible, rodents should be trained. Even though training is not always feasible, as much training as possible should be prioritized to enhance our understanding of how animal welfare can be improved through training.

S4F1.2

A Mouse Case: Micropipette-guided Drug Administration as Alternative to Oral Gavage

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[1]*University of Zurich, Zurich, Switzerland*

Abstract
Preclinical studies often require oral testing of compounds, traditionally conducted via oral gavage in mice and rats. However, this method poorly mimics voluntary human substance intake and poses risks of injury and stress to laboratory animals. This presentation introduces alternative approaches to oral gavage, focusing on the Micropipette-Guided Drug Administration (MDA) method (1).

The MDA method involves training mice to voluntarily ingest drugs mixed into palatable solutions, such as diluted sweetened condensed milk. This approach minimizes stress and improves the relevance of preclinical data by better simulating voluntary intake by humans.

The talk will describe the necessary training and examine the evidence supporting these alternative methods for delivering experimental substances, including analgesics, tamoxifen, and

others, while emphasizing the importance of animal welfare in research practices.

S4F1.3

A Rat Case: Voluntary Cage Change

N. Baumgart[1]
[1]*University Medical Center of the Johannes Gutenberg University Mainz/TARCforce3R, Mainz, Germany*

Abstract
The training of laboratory animals, particularly small rodents, offers promising opportunities to enhance both the quality of research and the welfare of the animals. Positive reinforcement training, a method that relies on rewarding desired behaviors, has been shown to be not only effective but also animal-friendly. This approach has the potential to reduce stress, improve the animals' socialization, and increase their overall well-being, which in turn can lead to more reliable and reproducible experimental results. By integrating positive reinforcement techniques into the daily care and handling of laboratory animals, researchers can foster a more cooperative and less stressful environment for both animals and handlers.

In our research presented here we specifically examined the use of positive reinforcement to train rats to voluntarily change cages, a task that is traditionally handled through more stressful methods. Rats are taught this behavior not only by the trainer but also through social observation, learning from the actions of their peers. Rewards such as treats or social interaction with the trainer can be used to reinforce the desired behavior, ensuring that the training process remains both motivating and stress-free and meets the requirements of the experimental settings. This approach is highly adaptable to various research settings, offering flexibility to meet the specific needs of different laboratory environments. Our findings suggest that this method not only enhances the animals' well-being but also provides researchers with a reliable and low-stress technique to improve the management of laboratory rodents.

S4F2.1

Effective Husbandry of Larval Zebrafish

J. Monteiro[1]
[1]*Champalimaud Foundation, Lisbon, Portugal*

Abstract
Zebrafish are a cornerstone in biomedical research. Because they are vertebrate with simple but representative systems, they strike a balance between scientific validity and ethical considerations, making them indispensable in research while adhering to the 3Rs framework.

In order to support accurate research outcomes, effective husbandry practices must be adopted during early life stages to ensure the health and growth of robust zebrafish colonies. This talk provides an overview of zebrafish larval rearing, focusing on practical examples of successful larval rearing protocols and innovative solutions. There will also be time for discussion of common issues. Key aspects will be addressed, such as:

- Environmental parameters
- Feeding
- Stocking density and minimum space
- Handling and tank cleaning

Workshop attendees will gain knowledge to troubleshoot common challenges and generate tailored protocols suited to the specific conditions of different fish facilities. These practices will ensure the fitness of zebrafish larvae, ultimately setting the foundation for high-quality scientific outcomes.

S4F2.2

Overview of Zebrafish Facility Management: Essentials for Care and Compliance

A. Borges[1]
[1]*GIMM - Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal*

Abstract
Managing a laboratory animal facility requires a multidisciplinary approach to ensure high-quality animal care, compliance with regulatory standards, and support for research objectives. This presentation will provide an overview of key components involved in effective facility management, with a focus on zebrafish and other aquatic models.

Key topics to be covered:

1. **Animal Care**: Establishing routine husbandry practices, health monitoring programs, and colony management strategies.
2. **Environmental Management**: Maintaining water quality, overseeing life support systems, and ensuring the infrastructure is well-maintained.
3. **Staffing and Training**: Recruiting skilled personnel, providing training, and supporting career progression to build a resilient team.
4. **Legal and Ethical Frameworks**: Addressing compliance with the EU Directive 2010/63/EU and promoting transparent reporting practices to enhance reproducibility and research integrity.

This presentation aims to highlight practical strategies for tackling common challenges in facility management, emphasizing the integration of technical expertise, ethical considerations, and operational efficiency.

By focusing on the unique requirements of zebrafish husbandry, this presentation aims to equip participants with essential knowledge to support their roles in research and facility operations, fostering a strong foundation for future growth and collaboration in the field.

S4F2.3

Basics of Aquatics Facility Design

B. Baur[1]
[1]*Aquaneering LLC, San Marcos, United States*

Abstract
As many research teams add aquatics models to their repertoire there is a growing demand for a basic understanding of the unique requirements of the design of these facilities. This presentation will cover standard aquatic lab design requirements.

Key topics to be covered:

1. Physical space/Layout
2. Housing and Life Support Equipment
3. System Options
4. Utilities

This presentation will cover space and utility requirements for aquatic research facilities small to large, as well as housing, life support, and accessory options. Special note will be made of differences between aquatic and rodent facility design requirements. Attendees will leave with a basic understanding of aquatic lab design.

S4F3.1

How to Effectively Talk About Your Work with Animals in Research

P. Clifford[1]
[1]*Americans for Medical Progress, Washington DC, United States*

Abstract
The future of biomedical research will be shaped by how animal research is perceived by the public. There have been decades of near silence on the topic by the research community, allowing animal research opponents sway public opinion. This is leading to anti-animal research legislative and regulatory initiatives based on misinformation, disinformation and lies.

As a result, more than ever before, we are now called upon to come together to change public attitudes and understanding about research that required animals. This session is designed to help participants gain the skill to talk about their work with animals proudly and effectively. We will be focusing on one-on-one conversations not formal outreach presentations, although many of the same principles may be applied. The learning experience will offer a starting point for participants to have conversations about animal research. It is important remember that practice will lead to confidence and effectiveness.

Objectives:

- Proudly talk with friends, family and/or acquaintances about one's role in animal research
- Understand ways to ensure effective communication
- Confidently answer questions related to animal research or address challenges

S4F3.2

Freedom to Speak: How Openness Contributes to a Culture of Care

J. Meredith[1]
[1]*Understanding Animal Research, London, United Kingdom*

Abstract
Working with research animals is socially valuable, important, and often difficult work. It should be a source of pride and satisfaction, and yet many people feel inhibited from talking about what they do because of concerns about being unfairly judged, misunderstood or abused. The pressure that this inhibition places on individuals can be deeply harmful to their peace of mind and general well-being, but is not often enough discussed in the context of the culture of care. This talk, based on UAR's long experience of working with animal research professionals to develop their public communications, will examine the obstacles to talking freely about animal research and explore ways to increase confidence in engaging with the public – in both formal and informal settings – to talk about why research animals are used, how they are cared for and how the harm that is sometimes involved can be ethically justified.

S4F3.3

Can We Talk? Communication between Animal Technicians and Researchers Is an Important 3R Facilitator

A. Milford[1] and E. De Clercq[1]
[1]*University of Basel, Basel, Switzerland*

Abstract
Animal technicians and those responsible for maintaining animal facilities are a cornerstone of the biomedical research sector, not only for their skills in animal husbandry, but for their deep compassion for animals, and their dedication and diligence. Communication between animal technicians or their managers with researchers can have a considerable effect on 3R implementation and animal welfare as a whole. This study draws on data from 18 interviews with animal technicians or animal facility directors across Switzerland. Through reflexive thematic analysis, we explore the facilitators and barriers to effective communication and outline the impacts of various approaches to communication on both animals and staff at animal facilities. Through these themes we develop recommendations to improve communication and laboratory animal facilities and thus improve 3R Implementation.

S4F3.4

Towards a Transparency Agreement on Animal Research in Greece

A. Xanthopoulos[1], G. Petrellis[2,3], I. Kyrmpizakis[1], E. Makri[1], M. Kalonikou[1], D. Lefkaditi-Koukeri[1], M. Havermans[2,4] and A. Tsingotjidou[5]
[1]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[2]*European Animal Research Association, London, United Kingdom*
[3]*Laboratory of Parasitology, Immunology-Vaccinology FARAH, University of Liège, Liège, Belgium*
[4]*Stichting Informatie Dierproeven, Hilversum, Netherlands*
[5]*Lab. of Anatomy, Histology and Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Abstract
National Transparency Agreements on animal research are important tools in the commitment to openness and transparency in the biomedical sector. Currently, ten countries have such a Transparency Agreements in place, supported by EARA (European Animal Research Association: https://www.eara.eu/transparency-agreements). They bring together researchers and communication experts to remove barriers towards open and proactive communication on animal research.

In the past, like in many other countries, animal facilities in Greece have been reluctant in giving insight into the use of animals in research. However, work by the Hellenic Society for Neuroscience and Pasteur Institute in Greece in collaboration with international organizations such as EARA has aimed at training the researchers for media presentation of their work and guiding them towards openness in animal research.

We are conducting a new survey among public and private institutions in Greece that use animals in research to assess motivation towards a Greek Transparency Agreement (TA). In this survey, we are asking the managers of the relevant facilities if they are willing to support the four commitments of the TA which refer to the promotion and improvement of internal and external communications by the institutions as well as the sharing of experiences and results.

Our aim is to create a proper environment for the development of a TA in Greece. Through our survey we will additionally select information on the different species used in research in Greece. Following this study, we will use this session to discuss the possibilities for a Greek Transparency Agreement.

S5A1.1

The Weight of the Culture of Care in Harm-benefit Analysis

J. Parks[1] and N. Verhave[2]
[1]*University of Southampton, Southampton, United Kingdom*
[2]*Rabdoud University Medical Center, Nijmegen, Netherlands*

Abstract
In the project evaluation the harm-benefit analysis is recognized by the inclusion of information on scientific goals and background versus the impact the procedures will have on the animals. Additionally, 10 principles are listed which are necessary to meet the requirements of article 38 (technical and scientific expertise) and 59 (positioning of the evaluating body) of the EU Directive. Number 10 is the knowledge of local culture and practices. In a formal evaluation on paper this principle may seem to be in conflict with some of the other principles.

Under the UK Animals (Scientific Procedures) Act 1986 (ASPA) the harm-benefit analysis is dealt with in section 2A (the principles of the 3Rs) and section 5 (the requirement for a harm-benefit analysis to be undertaken). Appendix I of the Guidance on the Operation of ASPA details how the harm-benefit analysis is carried out by the Inspectorate. The Animal Welfare and Ethical Review Body should submit project applications of a suitably high standard including all the information required to perform this analysis. This can be challenging!

Ten years of experience and discussion on project evaluation from different standpoints (The Netherlands and UK) will be reviewed, including which challenges were addressed, and which still need to be overcome. In this talk an overview is presented on how the review process can ensure that knowledge of local culture and practices is intertwined in project applications text and evaluation, even in the case where this is not anchored in the requirements.

S5A1.2

Fostering Ethical Practice in Academia: Before, during and Beyond the Life of a Project

N. Dennison[1] and S. Thomson[1]
[1]*University of Dundee, Dundee, United Kingdom*

Abstract
Full implementation of the 3Rs is critical in all projects to ensure the highest ethical standards are achieved. The competitive grant system within academia creates inherent challenges as animal studies can sometimes be viewed as "necessary" or "adding weight" to applications. The move to alternative methods can be viewed as "risky" compared to sticking with well published models and the system frequently means that the first opportunity that the Animal Welfare and Ethic Review Body has to comment on a proposed project is after the grant has been obtained, where the models to be used have already been agreed with the funding body. During the course of a project, time and money can be too limited to introduce or trial new models and publications at the end of a project often result in peer reviewers querying aspects of animal based studies and asking for additional data or repeat of work to address perceived shortcomings. In addition, poor reporting of studies can lead to issues with reproducibility.

However, there are many opportunities to address these challenges. Good communication and the opportunity to question applications before they are submitted, institutional recognition and support for non-animal methods, cross-disciplinary and between institute working, review of ongoing work within a project, challenge of reviewer comments and internal review and questioning of poor reporting practice are some of the ways that ethical practices can be introduced and implemented. Recognising and acknowledging that change is needed and working together to challenge norms can make a difference!

S5A1.3

Acknowledgement of Sentience in Project Evaluation and Animal Research Practice

K. ten Cate[1]
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Abstract
Time and again, we underestimate animals. Scientific research into animal capabilities, ethology, continues to provide us with new insights into what animals are capable of. Over the past few decades, science has shown us that animals, contrary to previous beliefs, are sentient beings, capable of experiencing suffering. Not only have more animal species been found to be able to suffer, but some species also exhibit various other abilities that may be morally relevant. There are species that can communicate (or even possess language), act intentionally, have individual personalities, and demonstrate culture and even a sense of justice. These are precisely the traits that have often been attributed exclusively to humans and are used as arguments to justify the belief that humans hold a higher moral status than animals, and that human interests therefore take precedence over those of animals. This is also the underlying normative (and, by derivation, legal) standpoint in many animal experiments, where the lives and welfare of animals are sacrificed to improve human health and welfare.

These new scientific insights from ethology however are (or should be) changing our perspective on animals, on what is important for them, and on what is morally justified in our treatment of them.

This presentation will reflect on the implications of these new insights on the capabilities of animals and will explore how we can better account for the interests of animals when evaluating project proposals and in the practice of scientific research involving animals.

S5A1.4

Promoting KARE: Kinship in Animal Research Ethics

J. Allen[1]
[1]*Trent University, Peterborough, ON, Canada*

Abstract
The value of alternative worldviews and reconciliation with Indigenous nations is increasingly being recognized by Canada. Alternative ways of knowing can offer improved options for management of the wicked problem that is the human relationship with other species. A survey to gauge the climate for change in how animal science is performed in Canada indicates this sector of work with animals is open to a significant evolution of current ethical standards and practices. Animal science is particularly well suited to being an early adopter of this ethic compared to other sectors of animal use because of a significant use of public sector funds and oversight.

Kinship ethics developed with Two-Eyed Seeing supports animal science beyond current stewardship practices; it also renews social license for animal research, which may decline if progress is not made in how animal science is performed. Responsibilities are based on a mutuality of being and ecological justice within a working relationship rather than strictly biology. The ethos offers animals and researchers shared value, reducing how extractive Western science can be through the practice of reciprocity. Kinship ethics also promote formal protocol at key points in an animal study to help promote adherence to the 3Rs teachings and reduce compassion fatigue in the industry. This can be done without significant added cost, administration, and does not compromise research goals or scientific methodology.

S5A2.1

The Laboratory Environment: A Neglected Factor in Implementing the SABV Policy in Mouse Research

I. Jaric[1], O. LaLoggia[1], J. Malikovic[2], J. Novak[1], M. W. Schmid[3], B. Voelkl[1], I. Amrein[2] and H. Würbel[1]
[1]*Animal Welfare Division, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[2]*Institute of Anatomy, Division of Functional Neuroanatomy, University of Zürich, Zürich, Switzerland*
[3]*MWSchmid GmbH, Glarus, Switzerland*

Abstract
The underrepresentation of female mice has led to the establishment of the Sex as a Biological Variable (SABV) policy, which mandates equal representation of both sexes in basic and preclinical biomedical research. However, the practical implementation of the SABV policy in mouse research facilities has raised questions about whether male and female mice should be housed in the same room or in separate rooms. Although rarely reported, this crucial aspect of the animals' environment can significantly influence physiology and behavior, thereby impacting experimental outcomes. Here, we examined the impact of housing male and female mice of two inbred strains with (mixed-sex housing room) or without (same-sex housing room) odor cues from conspecifics behavior, hippocampal molecular and structural plasticity, and sex-dependent biological function. The results showed strain- and sex-specific differences in hippocampal transcriptome and volume of the ventral hippocampus, which contributed to differences in exploratory behavior in the open field test. Additionally, female estrous cyclicity was affected by housing conditions, with females housed without male odor cues exhibiting suppressed or prolonged cycles, consistent with alterations in steroid hormone production. However, the presence or absence of odor cues from conspecifics did not affect the manifestation of aggressive behavior in male mice, but it did affect spermatogenesis. These findings suggest a complex interaction between housing conditions, genetic background, and neurobehavioral and reproductive outcomes, providing valuable insights for animal housing and experimental design, especially in studies investigating sex differences.

S5A2.2

Reduction by Design: Sex Inclusive Research Need Not Increase the Sample Size by Default

B. Phillips[1]
[1]Quantitative Biology, Discovery Science, R&D, AstraZeneca, Cambridge, United Kingdom

Abstract
A culturally embedded practice of studying only one sex in health research introduces a bias into the scientific knowledge base, and contributes to poor translation and irreproducible findings. It is also inefficient and unsustainable, resulting in an estimated overproduction of >25 million mice and rats globally [1]. Funders and regulators are driving change in the research landscape by moving from encouragement to mandates, with key bodies now requiring a justification for exclusion. However, progress is slow and barriers to the routine use of both sexes remain. Many of the perceived obstacles are rooted in statistical misconceptions and lead researchers to believe that inclusive designs will increase the number of animals needed and would thus be unethical and prohibitively expensive. Moving the dial to improve inclusive research practice requires a systematic approach to investigate and challenge these obstacles. In this seminar, I will share an in-depth examination of the consequences of including both sexes on statistical power [2]. I will demonstrate that there is no loss of power to detect treatment effects when splitting the sample size across sexes in most scenarios, providing that the data are analysed using an appropriate factorial analysis method.

S5A2.3

The Use of Rodent Models to Evaluate Sex Differences in Toxicological Risk Assessment

F. Maranghi[1], G. Lori[1], A. Tammaro[1,2] and R. Tassinari[1]
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[2]University of Rome 2 Tor Vergata, Rome, Italy

Abstract
In the frame of toxicological risk assessment – particularly hazard identification - the use of rodent models is crucial for understanding how chemicals affect biological systems [1]. The models should be as close as possible to the target population, including the specific variables as age, physiological status and sex. Rats and mice have been commonly used to evaluate the toxicity of chemicals since they: are easy to handle, maintain and cost-effective, have a well-characterized reproductive cycle, a short gestational cycle and an accelerated life span, huge databases of historical data. Indeed, the OECD Guidelines for the Testing of Chemicals, a collection of testing methods used for regulatory purposes, indicate rodents as preferred species.

Male and female rodents exhibit sex-specific differences in metabolism, hormone levels, tissue sensitivity, immune and neurobehavioural responses, leading to sex-linked susceptibility with a consistent impact on the severity and type of toxic effects; despite this, traditional toxicological studies often used males only, overlooking the potential differences between sexes, leading to incomplete or misleading evaluations. For instance, the presence and number of sex steroid receptors in target tissues may influence the response to chemicals in a sex-specific way. A particularly relevant example is represented by rodent models implemented to understand how the gender-affirming hormone treatments for trans and non-binary people may alter the susceptibility to certain environmental chemicals [2].

Incorporating sex as a biological variable is essential to advance toxicology research and improve risk assessments, ensuring more accurate safety guidelines for both male and female populations.

S5A2.4

Pain, Sex, and Death

J. Mogil[1]
[1]McGill University, Montreal, Canada

Abstract
Pain researchers have now come to some consensus regarding the existence of sex/gender differences in the sensitivity to and tolerance of pain in humans. In addition and more importantly, evidence is rapidly emerging that the sexes may differ *qualitatively* in their biological mediation of pain and analgesia. That is, different genetic factors, neural circuits, neuromodulators, and immune cells may be relevant to pain processing in males and females. I will make the case for the importance of sex-as-a-biological-variable policies as they pertain to pain, and then present several research stories suggestive of fundamental sex dimorphism in pain processing, the effects of pain on mortality, and the interaction between pain and social behaviour. These cases will illustrate how it is mandatory to account for sex in animal experimentation.

S5C1.1

Reduction of Surplus Animals With Breeding Management Software

P. Bugnon[1] and T. Buch[1]
[1]University of Zürich, Institute of Laboratory Animal Science, Zürich, Switzerland

Abstract
There are a number of factors that can help reduce the number of surplus animals. In fact, there are two sides to the coin: making better use of the animals produced and reducing the number of animals produced. Making better use of animals by, for example, using both sexes in animal experiments instead of just one sex, if this is not already being done. A better design that can reduce the sample size. But it is more difficult to figure out how to reduce the number of animals produced. Breeding management could make this possible, but it is a complex mathematical challenge. A few years ago, we started a major project with financial support from the Swiss 3R Competence Center 3RCC, which will enable us to calculate different breeding strategies for a breeding in order to find the one(s) that will

reduce the number of animals produced in the breeding steps before obtaining animals for an animal experiment.

S5C1.2

Do breeding practices impact the health and wellbeing of the mouse dam?

C. Boyle[1], A.S. Leuthardt[1], C. Calmbach[1], K. Walo[1] and G. Serra[1]
[1]*Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland*

Abstract
The female rodent breeder is the unspoken workhorse of animal research. Yet, current breeding practices are designed to maximize breeding productivity and have not been evaluated from the perspective of the health and wellbeing of the rodent mother, or dam.

In Switzerland, the vast majority of experimental animals are mice, with approximately 350,000 used yearly. We assume these mice were born to healthy dams, but have limited concrete data to support this. While reproduction is often viewed as a marker of welfare, is it truly sufficient evidence? When mouse breeding pairs are permanently housed together, the female breeder is often impregnated again hours after giving birth. The dam can continue this cycle of concurrent pregnancy and lactation for months, but the effect of this continuous physiological stress on her welfare is unknown. The boom in intergenerational research has demonstrated that various maternal and postnatal factors (e.g., maternal diet, litter size, time of weaning) can affect the wellbeing and health of rodent offspring. How these factors affect the dam during pregnancy, lactation and beyond is rarely reported. Based on available literature, we will review the known impact of pregnancy and lactation on physiological parameters in mouse dams, consider rodent breeding strategies in the laboratory in light of how mice breed in the wild, and identify what we still don't know about the amazing breeding mouse dam.

S5C1.3

Assessing the Health of Breeding Mouse Dams: Results from an Exploratory Breeding Study

A.S. Leuthardt[1], C. Calmbach[1], N. Prebianca[1], K. Walo[1], G. Serra[1], M. Gries[1], S. Botter[2], R. Palme[3], B. Tarigan[4] and C. Boyle[1]
[1]*University of Zurich, Institute of Veterinary Physiology, Zurich, Switzerland*
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[3]*University of Veterinary Medicine Vienna, Department of Biomedical Sciences, Unit of Physiology, Pathophysiology and Experimental Endocrinology, Vienna, Austria*
[4]*University of Zurich, Applied Statistics, Department of Mathematical Modeling and Machine Learning (DM3L), Zurich, Switzerland*

Abstract
In this 3R-based exploratory analysis, we are investigating whether breeding experience impacts the health of the mouse dam. Two commonly used inbred mouse strains [C57BL/6J; BALB/cByJ] were used to assess behavioral, metabolic, and nutritional endpoints in postpartum dams. The effect of the number of breeding cycles [1, 2, or 4] and the lactation duration [3 or 4 weeks] was investigated and animals were compared to age-matched non-breeding females [virgins].

Summary statistics demonstrates that maternal body weight and body length increases with the number of reproductive cycles regardless of the mouse strain. Further, we could observe a reduction in exploratory behavior with increase of reproductive cycles as well as expected strain differences. Fecal corticosterone levels, indicative of stress, varied before, during, and after pregnancy and lactation strain-specifically but were not influenced by parity or lactation duration. To assess mother-pup interaction pup retrieval and pup ultrasonic vocalizations were measured, which showed both no effect of parity and lactation, but profound strain differences. Differences in metabolic health investigated through indirect calorimetry, insulin sensitivity and oral glucose tolerance test will be addressed, as well as effects on nutritional endpoints through assessment of the effect of concurrent pregnancies on bone mineral density and plasma iron levels.

By conducting this comprehensive health assessment, we aim to understand how breeding experience impacts maternal health and wellbeing, with the goal of applying this knowledge to refine animal breeding for scientific use.

S5C1.4

Housing and Management Critical Points for Mouse Survivability in Breeding

G. Morello[1], B. Correia[1], S. Capas Peneda[1], S. Lamas[1], S. Brajon[1], A. Thomas[2], H. Wardle-Jones[3], M. Wiltshire[3], C. Gilbert[3] and I.A.S. Olsson[1]
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[2]*Wellcome Sanger Institute, Saffron, United Kingdom*
[3]*The Babraham Institute, Babraham, United Kingdom*

Abstract
Mouse pre-weaning mortality in breeding colonies is substantially variable and often high ($\geq$20%), a problem that goes directly against the Reduction principle of the 3Rs, and seems to be generally unknown across breeding facilities1. Our research has identified a few critical points associated with pup early mortality, including advanced dam age, unusually small and large litters, and litter overlap (cohabitating litters). Our study with >3300 C57BL/6J (B6) pups revealed that >60% and >10% of litters from trio (2F+1M)- and pair (1F+1M)-housed mice, respectively, are born in overlapped litters. While the mechanisms underlying pup mortality in overlapped litters are still unknown, keeping weanings on time may prevent some litters from being overlapped. Data from 591 B6 litters revealed that 9% of all weekday weanings were delayed by at least 2d, while 27% of litters ended up being weaned at least 1d past their wean target-day. Of these litters, 17% ended up being overlapped specifically due to the delay on weaning day. Our research has also indicated that adjusting the

mice microenvironment may improve pup survivability2. Cages in breeding rooms set for 24°C and 21°C weaned 7.6 ± 0.4 and 5.0 ± 0.5 pups per litter, respectively ($p = 0.03$). As the percentage of day 0 (parturition day) increased with nest at high temperatures (>27°C), the number of pups surviving increased ($p = 0.04$), demonstrating that it is possible to reduce mouse early mortality with minor changes in routine practices.

S5C1.5

Using Post-Mortem Analysis to Understand Causes of Pup Mortality

S. Capas Peneda[1,2], G. Munhoz Morello[1], J.-B. Prins[3], A. Olsson[1] and C. Gilbert[4]
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[3]*Leiden University Medical Centre, Leiden, Netherlands*
[4]*The Babraham Institute, Cambridge, United Kingdom*

Abstract
The laboratory mouse plays a central role in biomedical research, representing 50% of all animals used in the European Union (over 4 million annually). To align with the 3Rs principles—Replacement, Reduction, and Refinement—efficient breeding is essential to minimize animal use while meeting research demands. However,our research on the most commonly used mouse strain-C57BL/6J-reveals preweaning mortality (PWM) rates of up to 30% during the neonatal period (0–4 days post-birth). We have already showed that PWM is unaffected by inspecting breeding cages from birth,that infanticide is rare,and that first litters are not more susceptible to PWM than subsequent ones. Also,the presence of older litters in trios significantly increases PWM, though the underlying mechanisms remain unclear.

To better understand the causes of early PWM, we have developed a necropsy protocol for neonates aged 0–4 days. This protocol assesses stomach contents,evidence of breathing after birth, usage of brown adipose tissue, traumatic injuries and malformations. A total of 110 neonates,aged 0–2 days old,from three facilities in Portugal and the UK (F1: 57; F2: 25; F3: 26) were analyzed.

Preliminary findings indicate an incidence of traumatic injuries of 22% (some of which may have occurred after death),a stillbirth rate of 19%, with crucially only 8% of pups born alive having milk in their stomachs. Failure to suckle appears to be a major component of early PWM.

These results underscore the importance of post-mortem analysis in identifying the primary causes of neonatal mortality. Such insights are crucial for developing preventive strategies to decrease PWM.

S5C2.1

Handling and Training Protocols for Camelids in Research

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[1]*Sanofi Ghent, Ghent, Belgium*

Abstract
New World camelids, particularly llamas and alpacas, have gained significant prominence in biomedical research due to their unique heavy chain-only antibodies and derivative NANOBODY® molecules. This increased utilization has highlighted the critical need for comprehensive knowledge regarding the training and handling of these camelids in experimental settings.

At Sanofi Ghent, we have developed an effective handling and training program specifically tailored for camelids in research. Our approach synthesizes insights gathered from various sources, including specialized conferences, consultations with camelid breeders and veterinarians, and in-house experience.

Our protocol begins with an overview of camelid natural behavior and associated characteristics in research environments. We then detail our handling methodology, encompassing:

1. Effective techniques for catching and haltering
2. Best practices for managing camelids during veterinary examinations and blood draws
3. Strategies for safely relocating camelids (between stables and meadows) *throughout the facility*

A key component of our program is the intensive training regimen designed for young camelids. Unlike traditional farm animals, camelids require extensive habituation to human interaction. Our step-by-step training approach yields camelids that are significantly easier to handle, experience reduced stress levels, which benefits care and welfare.

This comprehensive protocol not only improves the quality of research outcomes but also prioritizes animal welfare and handler's well-being aligning with the highest standards of ethical animal research practices.

S5C2.2

The Sunshine Vitamin: Myth or Reality of Vitamin D Role in Marmoset's Welfare

R. Vaicekauskaite[1], C. Gandon[1], A. Calvo[1], P. Ance[1], A. Carbajal Brossa[2], M.A. Lopez Bejar[2] and P.-H. Moreau[1]
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Abstract
Despite the importance of welfare assessment in non-human primate research, there is little consensus on what should be measured for Common marmosets (*Callithrix jacchus*). Welfare assessments should include both environmental and animal-based indicators, evaluating both positive and negative states. Social behaviours like play, grooming, and exploration, are often suggested as indicators of positive welfare, whereas not been yet

fully validated. Nutritional needs, especially vitamin D3, are also crucial for marmosets. Access to sunlight or UVB light helps preventing vitamin D deficiency, yet many marmosets under human care are housed indoors with limited access to sunlight.

Therefore, we conducted a pilot study aiming to investigate UV lamp effect. We selected three pairs of marmosets with vitamin D deficiency and added UVB lamps to their environment, without altering their regular diet. The hypothesis was that two hours of UVB per day for one month would increase blood levels of vitamin D and improve animal welfare. Well-being was measured through behavioural observation and level of cortisol in hairs and faeces.

Preliminary results showed that UVB exposure increased vitamin D levels by up to 80%. Hair and faecal cortisol levels also increased under UVB treatment, with younger individuals showing higher levels. Activity, foraging, grooming, and social behaviours were higher when UVB lighting was provided, while alert and stressful behaviours decreased. Food consumption also increased significantly in the afternoon.

In conclusion, our findings support previous research, suggesting vitamin D as a critical welfare indicator for Common marmosets, affecting both physiological and behavioural well-being.

S5C2.3

Optimizing Rabbit Gavage Techniques through Data-driven Refinements

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Abstract
Oral gavage in rabbits is a commonly used technique for toxicological assessment of pharmaceutical compounds, especially in developmental toxicity and teratology studies. Unfortunately, due to physiologic and anatomic structures, oral gavage can be associated with higher mortality than other species due to perforations of the trachea, lungs, or esophagus. To identify what factors were associated with poor animal outcomes, we used a chi-squared test to analyze rabbit gavage outcome data across 10 sites for 7 different factors including: animal size, volume limit, number of people to administer a gavage dose, restraint positioning, tube type used, if a flush was used, and the average number of animals per doser. Three factors, (1) number of people to administer a gavage dose, (2) restraint position, and (3) if a flush was used were found to have a correlation with poor outcomes. Specifically, there was an increased risk of death when a single person both restrained and administered a gavage dose, and/or the animal was placed in dorsal recumbency, and/or a flush was not used following the test compound gavage. Knowing this information, we were able to enable the different sites to make changes to their technique to help improve their practices and minimize adverse outcomes with gavage dosing in rabbits.

S5C2.4

Unique Aspects of Naked Mole-Rat Care

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Abstract
Naked mole-rats (Heterocephalus glaber) are eusocial rodents with remarkable physiological characteristics, making them valuable models in biomedical and behavioral research. However, their unique social structure and adaptation to the environment present special challenges for animal care. We observed key aspects of naked mole-rat care, focusing on colony dynamics, eventual queen succession, which are critical to maintaining healthy and productive colonies, and removal of individuals is a critical factor for group stability.

The findings are based on observations and protocols established over several years in captive colonies. Colony establishment procedures, including selection of individuals for experiments and their reintegration into the colonie, were described in detail. The focus is on environmental conditions and their impact on colony health. The process of queen founding and factors influencing queen fall ("queen succession") were investigated using behavioral data.

The effective care of naked mole rats requires an understanding of their complex social hierarchy, where queen dominance strongly influences colony cohesion and reproduction. Queen succession often leads to temporary instability that requires careful monitoring. Experimental designs must take colony dynamics into account to avoid confounding effects on study results. Establishing new colonies from individual animals requires a high degree of tact. The knowledge gained will help to optimize animal welfare and ensure the reliability of experimental results.

S5C2.5

The MI-RAT, a Novel Murine Model for the Study of OA

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Abstract
Osteoarthritis, the leading cause of chronic joint pain, is studied through different animal models, but none of them is ideal in terms of reliability, reproducibility and translational value. The osteoarthritis rat model has been used more extensively in the past years because it offers structural and functional pain measure outcomes. However, there are a plethora of models available. There is no consensus on which is the most be translational model The surgical models that are the most used are destabilization of the medial meniscus (DMM) and anterior cruciate ligament transection (ACLt). The objective of the first study was to evaluate the structural, functional and molecular (spinal neuropeptides) outcomes of 3 murine surgical models of osteoarthritic pain

(DMM, ACLt and DMM/ACLt) and 1 chemical model (MIA). The DMM, ACLt and MIA models induced temporary alterations but not convincing structural changes. The DMM/ACLt combination induced more persistent functional alterations, correlated to structural and molecular changes.[1] Subsequent studies using this model confirmed the influence of sexual hormones, particularly estrogen, in pain control, and the necessity to work on ovariectomized females.[2] The introduction of calibrated regular exercise to the DMM/ACLt led to the Montreal Induction of Rat Arthritis Testing (MI-RAT) as being translationally validated for homogenous structural alterations, and corresponding functional and molecular pain signals,[3] including epigenetics.[4] Moreover, the application of quantitative sensory testing to MI-RAT offers a non-invasive assessment of facilitatory/inhibitory pain processes.[4] In conclusion, these recent development and validations of the murine MI-RAT enlarged our perspectives in a refined translational osteoarthritis model.

S5C2.6

Large Colony Housing of Rats in Drug Development

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Abstract
In drug development, rats are used to investigate the efficacy and safety of drug candidates. However, housing standards in small groups and cages limit their ability to exert their species-specific socialization and exploratory behavior that limits rats' welfare and the collection of translational data.

Diseases such musculoskeletal can significantly be influenced by social interaction and roaming activity that largely depends on the environment. For example, the progression of osteoarthritis (OA) is modulated by locomotion activity and in turn, spontaneous locomotion can reflect symptoms. For the pharmacological characterization of OA drugs, we developed a colony cage comprising four vertically connected levels including an activity tracking system for up to 48 socialized rats. While rats showed improved cooperativity, curiosity, body-mass index and less stress, the tracking system enabled objective health monitoring and symptom assessment. In OA-models, we found that RCC housing improved the relevance for the human disease and removed behavioral differences as an etiology of variation in structural changes.

In toxicology, rats are the default rodent species. The study design of single or repeat dose toxicology studies are prescribed by guidelines (e.g. ICH) including information on treatment groups, dosing frequency and observation schedules. Under consideration of these guideline requirements, the experiences made in pharmacology studies and the intension to improve rat welfare, we developed and characterized a novel Tox-RCC that comprises 4 type IV cages flexible connected horizontally. We found it's suitable to socialize 15 rats in a 4-week repeated dose toxicity study design with benefits for animals and data.

S5C3.1

Experiences and Challenges Supporting Scientific Community: What we Have Done What we Will Do

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Abstract
The Fondazione Guido Bernardini (FGB) is a non-profit organization focused on continuing education and training of professionals involved in the care and use of laboratory animals. Founded in 2009, FGB develop scientific events and training courses to improve continuous education in LAS, by a strong commitment of Scientific Committee members and the support of renowned speakers and lecturers.The mission to serve the scientific community, sharing knowledge and experiences, is the key to success, to stand by scientists, veterinarians, technicians, managers. Three main features characterize, basically, FGB activities and courses: the constant revision and update of the topics and learning outcomes, the time dedicated to extensive discussion sharing also individual experiences, the interaction between the participants and the speakers. The path was, and is, plenty of satisfaction and results but also challenging andsometimes not easy to face. Just as an example during the COVID-19 pandemic the FGB standard approach to in-presence courses and training was completely revised and new online training programs were developed, to keep up the offer of high-quality and safe training. This dramatic experience had an added value to revise and change our educational offer, our training activities opening new scenarios, exploring new opportunities.At time we have an annual blended portfolio (face-to-face and e-learning events) to keep offering a continue global learning. It will be expanded and in the future we will bring more and more opportunities to stand by and support the international scientific community.

S5C3.2

Achieving Competence in Laboratory Animal Science: A Pathway to Animal Welfare and Scientific Excellence

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Abstract
Achieving competence in laboratory animal science (LAS) is essential for improving animal welfare and ensuring high scientific standards. This competence is grounded in comprehensive education, rigorous training, effective assessment methods, and continued professional development (CPD). Beyond humane animal use and procedural skills, LAS competence requires ethical responsibility and a commitment to the principles of the 3Rs. CPD programs based on educational and training activities provide structured, lifelong learning opportunities, ensuring that personnel involved in animal care and use continuously improve their knowledge, skills, and professional behavior. Modern CPD frameworks incorporate modular education, blended learning

techniques, and species-specific training to address the dynamic requirements of LAS professionals. These robust CPD strategies promote animal welfare, encourage scientific quality, reinforce ethical standards, and enhance public trust in animal research.

S5C3.3

Animal Facility Management, (Too) Many Balls to Juggle

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Abstract
The animal facility manager is expected to protect animal welfare, keeps staff happy, facilitates research, keeps the leadership of the organisation out of trouble, stays within budget, ... In short, the facility manager is expected to serve many masters, who's objectives are not necessarily aligned. Even though the common theme in an organisation is that 'we are all pursuing the same goal, which is the general interest of the organisation'. Not seldom, the animal facility manager may experience the pressure of having to meet the capacities of the proverbial 'five-legged sheep'. A logical combination of training and contact with colleagues in similar positions is a way to resist that pressure. Specific courses on lab animal management and welfare are offered by different stakeholders including FGB. Even better, a comprehensive modular curriculum covering all aspects of animal facility management is in the making and will be on offer soon.

S5C3.4

Application of Lean Management in Animal Facilities: Pros and Cons

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Abstract
Lean is a management philosophy derived from the Toyota Production System, aimed at eliminating waste by focusing on activities that create value for the end customer. Originally developed in manufacturing, this approach has proven effective in optimizing processes across various fields, including laboratory animal facilities.

The author manages a 600 m^2 mouse facility housing approximately 6,000 ventilated cages dedicated to breeding genetically modified mice and conducting experimental activities. In 2011, Lean principles were introduced to reorganize animal care activities and improve operational efficiency. The initial transformation focused on cage and bottle workflows, balancing operations and aligning processes with autoclave throughput. Visual procedures, new layouts, and revised schedules were implemented, yielding striking results.

Over the following years, the Lean approach was expanded to additional areas, such as breeding management, health checks, and daily cage care. This continuous implementation resulted in a comprehensive transformation of the facility's operations.

Despite its success, the adoption of Lean Management also posed challenges, including staff adaptation and the sustainability of improvements over time. These experiences highlight the importance of fostering a culture of continuous improvement and ensuring staff engagement throughout the process.

By sharing the pros and cons encountered during this journey, this work offers valuable insights into the application of Lean methodologies in laboratory animal facilities, demonstrating both their transformative potential and the challenges they present.

S5D1.1

There is only One Welfare

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Abstract
While our institutions are facing increasing and legitimate demand of Culture of Care standards from citizens, collaborators and, eventually, customers, it is crucial to ensure that the welfare of our animals does not overwrite the well-being of those who take care of them. The One Welfare approach is then essential for balancing the needs of animals, humans, and the environment. This approach emphasizes the importance of considering all aspects of welfare when making decisions that impact the lives of animals, the people who care for them and their environment. Ultimately, prioritizing One Welfare ensures a more holistic and compassionate approach to animal care and management.

This presentation will show through concrete examples, how, by adopting a One Welfare approach, we can address the complex interactions between animals, humans, and the environment, and strive for solutions that benefit all parties involved. By prioritizing the interconnectedness of these elements, we can work towards creating a more harmonious and sustainable relationship between humans and animals. This sustainable perspective that navigates through the concepts of 3R, Culture of Care, openness, compassion fatigue and resiliency building, encourages us to consider not only the immediate needs of animals, but also the long-term implications of our actions on their welfare and the overall mental and physical health of the full animal research ecosystem.

S5D1.2

Developing a Training and Wellness Program to Promote a Culture of Care

S. Rosati[1]
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Abstract
At the University of Pennsylvania in Philadelphia, PA, we have implemented a training and wellness program for our university laboratory animal resources staff and scientific community. In this discussion, I will share the infrastructure we developed to have 3

successful programs; staff training, scientist training, and wellness. These programs all focus on the culture of care: caring for animals, caring for people and caring for ourselves. See how all 3 areas intersect and take a deeper look into each program to learn more during this oral presentation. I will share the fundamentals and goals of all 3 programs, as well as what it takes to start designing and implementing similar programming at your institution. In addition, I will present data collected over the last 3 years showing the effects of incorporating staff wellness programing as a norm in your animal program. This presentation will be a thorough overview of staff training, scientist training and wellness at the University of Pennsylvania and attendees will walk away with practical knowledge and tips to enhance their existing programs or create new programs in training, education and wellness that focus on the importance of the culture of care in the laboratory animal research industry.

S5D1.3

Empowering Caregivers, Building Resilience and Compassion through Empathic Listening

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[4]*University of Guelph, Guelph, Canada*

Abstract
Providing compassionate and humane care to research animals' hinges on the mental and emotional well-being of their caregivers. Recent studies highlight the prevalence of compassion stress and fatigue among Laboratory Animal Professionals. These findings emphasize the need for practical strategies to mitigate these challenges while enhancing resiliency and compassion satisfaction. Although emotional well-being is multifaceted, there are certain factors that are within our sphere of influence. A feeling of social connectedness, belonging, and community relies on how people relate to others; the relationship's quality and nature of mutual trust and benefit. One way to increase this sense of belonging is the ability to talk openly and empathetically with peers about the emotional toll of their work. Peer counseling can play a vital role in enhancing employee well-being, resiliency, and compassion satisfaction. Empathetic listening is a cornerstone of this peer support, which fosters a caring workplace grounded in mutually beneficial relationships and strengthens the overall Culture of Care.

Charles River Laboratories has implemented a scalable and sustainable approach to developing empathetic listening skills as a tool for peer support in the vivarium. We will explore the latest research on Emotional CPR©, an evidence-based method for connecting, empowering, and revitalizing peers through real-world scenarios within vivarium settings. Attendees will learn from our successes and challenges in building our Compassion Science Program, while providing actionable insights into building a Culture of Care, supporting the mental health of caregivers, and enhancing outcomes for both people and animals.

S5D1.4

Developing a Two-tiered Conversation Guide on Compassion Fatigue and Resilience in Mice Breeding Teams

F. Nunes[1] and L. Hallengren[1]
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Abstract
Compassion fatigue is a significant issue in mice breeding teams due to the associated ethical challenges, workload, and emotional bonds with laboratory animals. Addressing it is crucial for staff well-being and resilience, optimal animal care and promotion of welfare compliance.

We created a two-tiered conversation guide to foster resilience and mitigate compassion fatigue both in team and individual discussions. It consists of two components: (1) a **frequent-use guide** for regular discussions within teams or with individual team members, and (2) a **yearly reflection guide** intended for start-of-the-year meetings. These guides aimed to address three core stressors in mice breeding teams: (1) ethical challenges and emotional toll tied to culling surplus animals, (2) pressure associated with high workload and workplace demands, and (3) impact of emotional attachment to animals to encourage open communication and mutual support.

The frequent-use guide addresses daily stressors and promotes resilience through regular dialogue. It includes sections on: (1) normalizing conversations about emotional challenges, (2) identifying and addressing "micro-stressors", (3) fostering peer support, and (4) encouraging self-care practices. The yearly reflection guide helps teams review the past year's challenges and achievements, identify recurring stressors, and share personal resilience strategies for the coming year.

A pilot implementation of structured conversation guides was conducted, with anonymous surveys used to assess their impact on compassion fatigue and resilience. This dual approach provides flexibility for immediate and long-term needs. Future work will refine the guides based on feedback and expand their use to mice breeding teams in other facilities.

S5D1.5

Lab Life through the Lens: Portraying Animal Research in Video Format

R. Miller[1,1] and M. Harvie[1]
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Abstract
A handful of pictures or a short video can do more to dispel misconceptions about the conditions and treatment of animals in labs than thousands of words. But video content doesn't have to be reserved for only public facing communications. Videos are a powerful training resource and can also be used to record animals on study when discussing welfare concerns with colleagues.

This talk will be presented by Understanding Animal Research's video producer, Robert Miller, as he shares his insights of filming animals in research and considers the importance of visual storytelling. The talk will explore how film can

support a culture of care through improved transparency in public communications and the creation of more powerful training materials for internal audiences. Many video examples will be featured and discussed, demonstrating the best practice for capturing animal research on film. Ultimately, the aim is to provide audience members with tips and tricks that will be useful for creating powerful videos.

S5D1.6

Animal Facility Building Management - Thinking Inside the Box

C. Bergmann[1] and M. Leach[2]
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Abstract
Adequate estate buildings and infrastructure are critical to ensuring that animal facilities maintain safe, stable, and appropriate environmental conditions across all species.

Variable environmental conditions, including airflow, temperature, and relative humidity, can have a detrimental effect on animal physiology, increasing variability and reducing reproducibility between facilities, as well as impacting animal welfare and human safety. However, in academia, Estates infrastructure, operations, and budget allocation are typically managed centrally, outside the control of animal facility management. The engineering department may have limited training, experience, and awareness of the unique requirements of animal facilities and how to deliver safe and resilient operations. The higher education sector is particularly vulnerable due to unpredictable research income and variable student recruitment, which may impact the ability to maintain and invest in facilities and plant.

Here, we describe the practical challenges, risks, and solutions associated with the management of estate facilities in academic research settings in the UK. Positive stakeholder engagement, consideration of appropriate line management, and effective communication are critical. A strategic approach between academic leadership and animal facility management is also essential to ensure long-term investment to support the desired academic research portfolio.

In this presentation, we describe an innovative, solution-focused framework to bridge the potential gap between building management and facility operations. The framework is designed to support AWERBs in self-assessing their internal compliance with estate building management and driving continuous improvement. It offers an inclusive, team-based approach to ensure regulatory compliance.

S5D2.1

The EU Education & Training Framework 2.0

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[2]*LASA, London, United Kingdom*
[3]*Department of Health, Northern Ireland, Belfast, United Kingdom*

Abstract
The EU Framework for Education and Training was one of the first guidance documents developed under the Directive. It laid out the basic quality structure for modular training that can be tailor-made to serve for different needs to make training more efficient and flexible.

Much progress has been made since then. The focus has shifted from basic requirements to acquisition and demonstration of competence and continued professional development. The experience gained from working under the Directive has highlighted the importance of some key roles. Modern tools have emerged, transforming the way we acquire and demonstrate competence, making it more effective and accessible.

In response to these developments, the European Commission, Member States and stakeholders have collaborated on a comprehensive revision of the framework. In this talk, we will present the key changes and new elements proposed for incorporation in the framework, ensuring that the framework remains at the forefront of competence development in the Union.

Close to 20 of the agreed modules have already been transformed into easily accessible, free-of-charge eModules – benefiting both individuals and course providers. These include innovative eModules for competence assessors, inspectors, and designated veterinarians, building on the success of the previously released eModule for project evaluators.

The revised framework, supported by new resources based on commonly agreed quality criteria, lays the groundwork for the mutual acceptance of competence claims, fostering the free movement of researchers and care staff within the Union.

S5D2.2

Educational Resources and a New EU Manual for Animal Caretakers

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Abstract
Education and training are crucial for advancing scientific competence and promoting ethical research practices. To achieve these goals, the European Commission, with funding from the European Parliament, has developed a suite of versatile educational tools based on the Three Rs principles (Replacement, Reduction, and Refinement). These resources span all educational levels, from primary schools to universities.

A key initiative supported by EU Schoolnet introduces primary and secondary students to the Three Rs through engaging learning scenarios and interdisciplinary activities. For students aged 14–18, an innovative open-access virtual reality (VR) application provides a fun and effective way to explore alternative research methods by simulating laboratory experiments.

For higher education, EURL ECVAM has convened experts to devise strategies for incorporating the Three Rs into university curricula and expanding professional development through a "train-the-trainer" approach.

Additionally, a comprehensive EU caretaker's manual has been developed to support entry-level staff. This resource offers essential guidance covering all key areas of this role promoting high standards of animal care and welfare. By making this manual freely available, it is hoped to become a go-to resource, a trusted companion, in every animal facility.

This extensive suite of educational tools not only supports the development of scientific skills but also fosters a culture of care and ethical practices in laboratory animal science and beyond.

S5D2.3

The European CPD Framework

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Abstract
The European Directive (Article 24, paragraph 1, point c) requires that all breeders, suppliers, and users are required to have a named person/(s) responsible for ensuring that the staff are adequately educated, competent, and continuously trained and that they are supervised until they have demonstrated the requisite competence.

This directive highlights the importance of maintaining and continuously improving the competence of all individuals involved in the care and use of animals for scientific purposes. Achieving initial competence is not sufficient; ongoing professional development is critical to ensure high standards of animal welfare and the reliability of scientific outcomes.

Currently, there is no harmonized framework to support Member States in implementing a structured and flexible approach to Continuous Professional Development (CPD) that aligns with the varying systems already in place or under development in different countries. This lack of standardization represents an opportunity for improvement to enhance the free movement of professionals within Europe and beyond.

To address this, the ETPLAS Preparatory Action working group proposes a comprehensive CPD framework. This initiative aims to provide a clear structure for Member States, facilitating the formalization of CPD for individuals engaged in laboratory animal science. By fostering skill development and recognition, this framework supports the mobility of professionals, ensures compliance with the directive, and promotes the consistent application of best practices across Europe and internationally.

S5D2.4

FELASA Recommendations for Continuing Professional Development for Professionals Involved in Animal Research

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[6]*Institute of Laboratory Animal Science, University of Zurich, Zurich, Switzerland*
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Abstract
A continuing professional development (CPD) process for professionals involved in animal research should be implemented in all organisations to ensure that professionals keep up with new scientific knowledge and practice regarding animal welfare and laboratory animal science (LAS). These recommendations advise on a lean and transparent continuing professional development (CPD) process for keeping all functions, roles and tasks included in the Directive 10/63/EU competent throughout their professional career. CPD starts after basic training and when individual competence is achieved. The first step is to build up the CPD portfolio of the professional that will be regularly reviewed by the Person Responsible for Training & Competency (PRTC) in line with the professional's personal development plan (PDP). A structured portfolio for the recording and assessment of CPD that institutions should include in the PDP to enable CPD process monitoring of their collaborators is proposed; it will be the basis for regular competency review of personnel. We recommend such review every 5 year cycles maximum.

The question of the minimal amount of CPD a person should provide is discussed and recommendations are issued.

The paper discusses also the minimal information that each CPD certificate should mention in order to develop a common framework for assessing CPD activities across institution.

To conclude implementation of an harmonized CPD strategy, with planning and registering, will facilitate mutual recognition and movement of personnel. Moreover, promoting high-quality CPD motivates and rewards staff and will promote a culture of care.

S5E1.1

Advancing Preclinical Animal Model Selection with AI: A New Frontier for Laboratory Animal Veterinarians

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Abstract
Effective animal model selection is essential in preclinical cardiovascular research, yet current methods relying primarily on body weight can result in complications, increased severity, and inefficient animal use. Here we explore how artificial intelligence (AI), combined with detailed cardiac morphometric data, can optimize model selection, reduce severity, and advance the 3R principles.
Methods & Results: Cardiac CT scans of 24 Swiss Large White pigs were analyzed, measuring key parameters such as aortic valve diameter, mitral valve commissure-to-commissure (C-C) and septo-lateral (S-L) dimensions, tricuspid valve size, pulmonary valve diameter, left atrial height, and coronary ostium heights. Animals were grouped by body weight (50–60 kg, 80–90 kg, and 100–110 kg).

Measurements demonstrated significant anatomical variability. Aortic valve diameter ranged from 23.55 ± 1.42 mm to 29.86 ± 1.73 mm, pulmonary valve diameter from 18.42 ± 1.11 mm to 23.72 ± 1.47 mm, and left atrial height from 15.20 ± 1.25 mm to 20.30 ± 1.64 mm. Coronary ostium heights remained consistent, emphasizing the limitations of weight-based selection.

AI could transform these complex datasets into actionable insights, identifying optimal animal matches for specific devices, predicting procedural outcomes, and flagging potential risks—all with unprecedented precision.
Conclusions: By harnessing morphometric data in cardiovascular research, AI can refine selection processes, minimize mismatches, and enhance predictability. This avoids unnecessary animal use and accelerates innovation while adhering to the 3R principles. For veterinarians, AI represents not just a technological advancement but a paradigm shift, enabling a more ethical, efficient, and scientifically rigorous approach to preclinical research.

S5E1.2

A Dynamic Evidence-based Platform to Support Animal Model Selection in Cancer Research

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Abstract
Selecting an animal model for the testing of small molecules in targeted cancer therapies is critical for successful clinical translation. However, with >20.000 publications per year, selection is time- and resource-consuming. Therefore, we developed a Systematic Online Living Evidence Summary (SOLES) using automated tools, such as machine learning classification algorithms and text-mining tools to provide a dynamic and continuously updated synthesis of all the relevant evidence in this research field. The curated database includes automated annotations of various experimental characteristics such as the animal population, tumour model types and cancer outcomes, as well as study quality characteristics and relevant metadata (e.g., FDA approval of tested intervention). The results are implemented in an interactive web-based dashboard that can be used to filter and visualise studies based on features of interest.

Such living resources support evidence-based decision-making for selecting animal models with high translational validity that align with human outcomes. The Cancer-SOLES platform may be used by interested stakeholders (e.g., scientists, animal welfare officers) to identify animal models with high external validity and to optimise the preclinical study design. Further, it may be used to support the conduct of specific systematic reviews, and to identify evidence gaps, thereby minimising the conduct of redundant animal studies.

In this presentation, we demonstrate the first version of the Cancer-SOLES dashboard and aim to gather input from potential users and interested stakeholders on how to further develop this platform to maximise usability and utility. For this, an online survey link will be shared among conference attendees.

S5E1.3

Virtual Reality, a Novel Training Tool for Preclinical Research

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Abstract
In compliance with the 3Rs principles, developing new learning methods in the field of preclinical research is necessary to refine the training process for the acquisition of advanced technical skills and to reduce the number of animal use.

Virtual Reality (VR) technology is such an innovative teaching method and is currently emerging in the field of training tools for human clinical practice.

We will present an overview of VR, and of its practical applications including the example of the first immersive veterinary tutorial in VR which has been developed and used by our surgery team.

Immersive video is based on "first-person view" video (180 degrees, 3D, ultra-high definition) embedded in a virtual reality headset, which allows the development of learning by imitation, facilitated by an immersive and realistic environment, and completed with additional assets (blended learning). We have been using this tutorial both for initial training and for refresh training (maintenance of technical skills and best practices). Our data demonstrate that immersive video facilitates the initial training and increases self-confidence of learners which results in higher operator performance, compared to conventional teaching

strategies. Recent scientific studies have also shown that the use of this technology increases memory retention rate and results in fewer performance errors.

We will discuss the benefits and drawbacks of this innovative solution which is a useful and efficient training tool for highly skilled technical procedures, in surgery as in other disciplines and which also may help reducing the number of animal use before trainees' validation.

S5E1.4

Balancing Innovation and Accuracy: AI-Generated Clinical Score Sheets in Focus

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Abstract
In vivo experiments increasingly utilize clinical score sheets to minimize animal distress. A score sheet is a document containing a list of specific symptoms, behaviors, and intervention guidelines, designed to enable an objective clinical assessment of experimental animals. Artificial Intelligence (AI) technologies are being applied more frequently in preclinical research, not only for data analysis but also for documentation processes. In a previous study, we evaluated the performance of ChatGPT-3.5, ChatGPT-4, and Google Bard in generating clinical score sheets for animal welfare assessment. Data collection for that study was conducted at the end of 2023. Since then, AI and large language models (LLMs) have evolved exponentially, introducing new tools with enhanced capabilities. In the present assessment, we extended our evaluation to include newer AI systems, such as Claude from Anthropic and Gemini from Google. This study aims to compare the performance of these advanced models with earlier LLMs in generating precise, structured, and reliable clinical score sheets.

Despite demonstrating notable improvements over the previous study, the findings reveal a duality in AI-generated score sheets: although some models consistently provide well-structured outputs, all exhibited variability in assigning numerical values to symptoms and defining intervention thresholds accurately. The study also identified the impact of the predominance of North American versus European literature on the AI-generated score sheets. These results suggest the importance of integrating AI-generated tools with expert review to ensure precision and reliability in using score sheets and other documents generated by AI for the animal research license process.

S5E1.5

The Power of Synthesizable Data: Strategies for Maximizing the Impact of Research

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Abstract
Research synthesis methods, including systematic review, are increasingly used to integrate and evaluate evidence from animal experiments. Systematic reviews are considered an animal-free innovation as they lead to new results and insights without needing additional animal studies: extracting information from existing data. Evidence from systematic reviews can directly inform 3Rs-related experimental decisions to focus and prioritize future animal experiments, and help prevent unnecessary duplication by comprehensively determining when research questions have been sufficiently answered. Ensuring primary data, generated from animal experiments, is synthesizable increases scientific value from animal experiments by enabling researchers to effectively synthesize and build on existing knowledge, accelerating generation of new insights. Here, we outline how primary research contributes to systematic reviews and how to make animal research "synthesis-ready".

We introduce tools and practices to facilitate designing and conducting synthesis-ready research. We present pre-registration of animal experiments, which protects the researcher against concerns of selective results reporting, enabling checking concordance between the methods as planned and executed, to ascertain whether discrepancies are addressed. We present the principles of open and FAIR data, highlighting how open datasets and making individual animal data machine-readable facilitates meta-analysis. We emphasize the importance of reporting animal research in a transparent manner, facilitating a clearer understanding of experimental conduct and enabling exploration of between-study variability. At each step of the process, we share resources and practical tools to improve the impact of primary research on decision-making. We conclude with future perspectives on embedding evidence synthesis into the research cycle, benefiting all scientists.

W1B1

Severity Classification and Reporting Under Directive 2010/63/EU - A FELASA Train the Trainer Workshop

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[4]*LASA - Laboratory Animal Science Association, London, United Kingdom*
[5]*Radboudumc, Nijmegen, Netherlands*
[6]*Centre de Recherche en Neurosciences de Lyon, Lyon, France*
[7]*ASNR - French Authority for Nuclear Safety and Radiological Protection, Fontenay-aux-Roses, France*

Abstract
Directive 2010/63/EU introduced the requirements for the classification of procedures during the application for project authorisation to use animals in scientific procedures, and to report the actual severity experienced by each animal. These requirements provide opportunities to refine the adverse effects of procedures.

Consistency in severity classification across Member States is a key requirement to promote harmonization in animal use and statistical reporting.

The FELASA Severity Workshops Core Trainers Group (SW-CT), who have developed additional guidance and examples of severity classification and reporting of well-established validated animal models used in scientific procedures, has designed Workshops on Severity Classification and Reporting Under Directive 2010/63/EU.

Since 2016, the SW-CT has ensured the organisation of over 70 workshops in 16 European countries, and at all FELASA Congresses. It remains clear at these workshops that there are still significant misunderstandings of the severity framework as set out in the Directive, and that further workshops would be helpful across EU Member States (MS) to continue to promote a consistent approach to severity assessment and reporting and, as a consequence, improve animal welfare, and promote a level playing field for the scientific community.

Currently, the FELASA Severity Workshops rely on a network of over 40 official Trainers. The continuation and expansion of this project relies on having sufficient suitable Trainers across Europe.

The main objective of this workshop is to train candidates to a high standard, such that they can organise and deliver FELASA Severity Workshops.

NOTE: Delegates should also attend session AS-FELASA-2025-00783 in the afternoon

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W1B2

FELASA Workshop on the Classification and Reporting of Severity of Procedures using Basic Models

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Abstract

Directive 2010/63/EU requires that experiments be designed to cause the least pain, suffering, distress or lasting harm to the animals used and requires that all procedures be assigned a severity classification in advance of the procedure being performed. Moreover, recent requirements for non-technical summaries ask for the inclusion of a 'best estimate' of the actual number of animals in each severity classification of a given project. Lastly, in the annual Statistical reports, each animal must be reported, based on the actual experienced severity. Severity classification is also key in determining whether or not an animal can be re-used in further procedures.

The implementation of a severity classification process, is thus a big challenge in animal studies. Having a very good understanding of the subject is paramount for properly evaluating research projects and to implement procedures with the least harmful effects on animals (refinement). Reporting of actual severity helps to refine similar procedures going forward and improves public transparency.

Consistency of severity assignment across Member States is of the utmost importance to harmonise project licensing and reporting. Therefore, the European Commission has encouraged FELASA to perform Workshops on the Severity Framework, including classification and reporting of Procedures, that could be held across EU. This Workshop will give you an introduction to what must be done, and prompt you to address severity on the three crucial steps where it must be considered: designing the project, monitoring its course and assessing the outcomes after it has ended.

W2A1

How to Teach Statistical Reporting of Genetically Altered Animals Within the European Union

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[3]*SPFCO-DGRI B5, Paris, France*

Abstract

Creation and breeding of genetically altered animals (GAAs) play a prominent role in Directive 2010/63/EU. Correct implementation of the Directive and accurate reporting of the uses of GAAs requires a combination of basic knowledge of GA animal management and a thorough understanding of the legal requirements, as there are many different categories in which these animals can be reported. The European Commission's effort to promote transparency in animal-based research is partly based on accurate data collected from the Member States. However, there is still room for improvement and harmonization of data quality. The legal framework and guidance documents provided by the Commission seem not yet to be fully understood and applied in practice., It is important that all involved in data collection and submission, including scientists, animal technicians, animal unit managers and project holders fully understand the requirements.

This workshop is designed to ensure individuals within Establishments who have some responsibility for training, gain the necessary understanding to enable them to accurately inform other relevant individuals of the reporting requirements. The objective is to promote an accurate and consistent approach to the reporting of GAAs, to disseminate guidance on this specific topic, and to clarify ambiguous areas. It will also provide a unique platform for identifying discrepancies in interpretation among Member States, which will assist in developing specific examples to be used in future workshops at the national level.

W2B1

Introduction to the RSPCA's Roadmap to Reducing Severe Suffering

P. Hawkins[1]
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Abstract
The RSPCA initiative 'Focus on Severe Suffering' began in 2012, and has enabled the scientific community to make significant achievements with respect to reducing and avoiding 'severe' severity in animal research and testing. A key resource from this initiative is the 'Roadmap to reduce severe suffering', which describes a practical, step-by-step process for reviewing animal's life experinces, so that refinements can be identified and implemented to the fullest extent. The Roadmap is free to download from the Focus on Severe Suffering website, which is also endorsed by the UK Laboratory Animal Science Association, Laboratory Animals Veterinary Association and Institute of Animal Technology.

This workshop will begin with a short presentation, followed by interactive group work to apply the Roadmap tool in practice to a realistic but feasible procedure, identifying opportunities to apply refinements throughout an animal's lifetime. The session will be of interest to scientists, animal technologists, veterinarians and ethics committe or Animal Welfare Body members. Although the parent project relates to reducing severe suffering, the Roadmap approach to understanding animals' experiences and identifying potential cumulative effects can be applied to any level of severity.

W2B2

Accuracy and Confidence in Assessment: Constructing DOPS in Practice

I. Tiebosch[1] and L. Whitfield[2]
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[2]OWL Vets Ltd, Mildenhall, United Kingdom

Abstract
The humane treatment of animals used in science entails, amongst other requirements, that they are used and cared for by competent staff. Competence is understood as established competency in a given professional activity, through education and training, experience, and continuing professional development[1]. Establishing competency in procedures involving animals warrants trust that one is competent in caring for, handling, and carrying out said procedures competently. To establish trust in this regard is not easy, for it needs clarification that the candidate assessed not only holds the knowledge, but also the right skills, and attitudes, and shows care for and empathy towards animals. Direct Observation of Procedural Skills (DOPS), is a method used in medical education to assess the practical skills of healthcare professionals as they perform them, and has been adopted in laboratory animal science[2]. Indeed, DOPS are nowadays an important part of competence-based assessment.

The basis of a DOPS is an assessment sheet. When constructed and used correctly for both formative and summative assessment, it can allow measuring knowledge, practical skills and attitudes. Constructing them is an iterative process with several steps, which in itself harmonises the assessment while increasing the validity and objectivity of the assessment strategy3. Before constructing DOPS it is recommended to learn the different options, strategies and pitfalls of the process, which involve not only competence assessors but supervisors as well. Well-designed DOPS sheets can help ensure that animals used in science are always properly handled and cared for by established professionals.

W2B3

End to End Severity Reduction: From Generation to Colony, Refining GA Techniques and Practices

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Abstract
In recent years, producing new genetically altered (GA) strains has largely relied on genome editing technologies such as CRISPR. These techniques are highly efficient and provide an opportunity to reduce the number of animals required to generate new strains, however with the efficiency, comes an increased risk of introducing new mutations in ways that increase severity. This workshop will follow the process of generating new mouse strains via genome editing, highlighting the areas of increased risk in terms of animal welfare (such as introducing debilitating mutations) and discussing ways of reducing the impact on the resultant animals.

The group will then discuss, with actual examples, the issues around establishing and breeding GA mice with a focus on genetic integrity, quality control, welfare assessment, appropriate humane endpoints and challenges of balancing justification of biomedical research with animal welfare.

W2C1

European College of Laboratory Animal Medicine Workshop: Vets Present your Case!

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[2]Merck RBM, Colleretto Giacosa (TO), Italy
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[4]University of Fribourg, Fribourg, Switzerland
[5]Labcorp, Huntingdon, United Kingdom

Abstract
The European College of Laboratory Animal Medicine designed a workshop for veterinarians who work or wish to work in the field of laboratory animal science and medicine. The workshop was inaugurated during the 2025 FELASA Congress. The workshop aims to address key points of veterinary advice offered by laboratory animal veterinarians while performing their designated

veterinarian or clinical veterinarian duties. An introductory presentation entitled "Veterinary consultation on laboratory animal care and use" (Argyro Zacharioudaki, Arianna Aricò) sets the framework of veterinary advice on animal care and use and underlines legal requirements and the stages of project design and implementation and overall establishment operation where the veterinarian can offer valuable input and oversight in order to ensure animal welfare. This is followed by group work on solving three selected real case-scenarios. During group work participants are asked to consider aspects of veterinary consultation and communication while advising for refinement and treatment. Group work discussions and "solutions" are presented to all participants at a final discussion facilitated by the veterinarian who shared the original scenario. For the first workshop, three cases were prepared: Case 1: "A mouse death in imaging" (Michael Wilkinson); Case 2: "Is the pathogen the culprit?" (Nina Eva Trimmel); Case 3: "What happened to the pig?" (Joana Almeida).

W2D1

Effective Mediation Strategies in Animal Research: A Comprehensive Workshop for All Stakeholders

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Abstract
Mediation techniques offer substantial benefits to staff and stakeholders in the complex field of animal research. These methods provide a structured approach to preventing and resolving conflicts, improving communication, and fostering collaboration within research environments. For animal care staff, researchers, veterinarians, and administrators, mediation can help address ethical concerns, streamline protocols, and enhance overall animal welfare. It allows for the balanced consideration of scientific objectives alongside animal well-being, often leading to more refined experimental designs. Furthermore, mediation can bridge gaps between different departments, reducing misunderstandings and promoting a culture of openness. For external stakeholders, such as regulatory bodies and animal welfare organisations, these techniques can facilitate constructive dialogue, ensuring that diverse perspectives are heard and incorporated into decision-making processes. Ultimately, the implementation of mediation in animal research settings can lead to more ethical, efficient, and harmonious practices that benefit both scientific progress and animal welfare. A real asset for a good culture of care.

Objective: Equip animal research professionals with mediation skills to enhance communication and foster a culture of care within their facilities.

Overview: Introduction to essential mediation techniques tailored for the animal research environment. Attendees will learn practical skills to facilitate effective communication, resolve conflicts, and promote a positive workplace culture.

Format: Interactive sessions combining lectures, role-playing exercises, case studies, and group discussions.

Outcome: Gain practical mediation skills to foster a more collaborative, efficient, and caring environment in animal research facilities.

Target audience: Animal care staff, scientists, veterinarians, members of ethics committees and animal welfare bodies, facility managers.

W2D2.1

The Elephant in the Room – Mistakes that Were, Are, and Will Be Made

C. Johner[1]
[1]*Max-Planck-Institute of Immunobiology & Epigenetics, Freiburg, Germany*

Abstract
Caroline Johner will mostly speak openly about specific mistakes she has personally experienced. Caused by various situations s.a. failure of equipment, miscommunication or incorrect animal interventions> these can have drastic effects on animals, people and research results. She will offer some preventive strategies with their opportunities and their challenges.

W2D2.2

Redefining Animal Welfare – The Impact of Critical Incident Reflection

S.J. Bischoff[1]
[1]*University Hospital Duesseldorf, Central Institution for Animal Research and Scientific Animal Welfare, Duesseldorf, Germany*

Abstract
Sabine Bischoff's presentation focuses on error management in laboratory animal science. Demonstrating the benefits of transparent error reporting for animal welfare, she will also share insights into CIRS-LAS.org database reports and strategies for implementing error culture in scientific institutions.[1]

W2D2.3

Scientific Benefits of Better Error Management

N. Drude[1]
[1]*BIH QUEST Center for Responsible Research, Berlin Institute of Health (BIH) at Charité, Berlin, Germany*

Abstract
This workshop encourages participants (animal caretakers, veterinarians, researchers, science communicators, etc.) to reflect on what is really holding back improvements in their work environments, and will address a range of possible solutions. Natascha

Drude will discuss the scientific benefits of improving error management in laboratory animal science, emphasizing how transparent error reporting can enhance reproducibility and preclinical-to-clinical translation. Drawing from real-life examples and hands-on experiences, she will illustrate practical strategies for fostering an error-resilient research culture. She will advocate for leveraging lessons from these experiences to stimulate a debate on implementing systemic improvements for robust, reliable science.

As Head of Responsible PrecliniX at the BIH QUEST Center for Responsible Research, N.D. strives to help others improve the quality and reproducibility of biomedical research. Her firsthand and secondhand experiences confirm that proactively addressing mistakes improves not only the welfare of lab animals but also scientific outcomes.

W2D2.4

Creating a Culture of Care and Learning through Open Reporting of Events

S. Robinson[1]
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Abstract
A systematic process for reporting observations and events that have the potential to a) help with continuous learning b) improve animal welfare and c) support staff; was developed (1). The process took learning from the safety, health and environment arena on accident prevention using Human and Organisational Performance (HOP) philosophy where human error is expected and normal. The key aspect of success was the learning approach to following up on events and observations. Underpinning the systematic process is the 'Learning from Observations and Events Log'. An environment was created that promoted continuous improvement for both animals and staff by recognising, rewarding and sharing good practice, as well as where near misses and events were openly reported and learnt from.

We recognised early on that using a system and learning approach to follow up on observations and events rather than a people and blame approach was key to developing open reporting and a positive culture. In the systems approach, errors are consequences rather than causes, having their origins in systemic factors.

W2D2.5

Mistaken Assumptions – How Public Science Communication Shapes Error Cultures in the Lab

F.A. Dehmelt[1,2]
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[2]*Tuebingen University, Tuebingen, Germany*

Abstract
The way we present scientific work in public can make our jobs easier – but it can make them harder, too. Florian Alexander Dehmelt will highlight some **unintended consequences of successful science communication**, and how to avoid them: An overly positive public image raises unrealistic expectations, setting us up for future failure. And an excessive fear of public backlash can leave genuine problems unaddressed, harming both our animals and the people who take care of them. He will argue that sensible science communication leaves room for nuance and complications, and rewards those who reveal them.

F.A.D. is a neuroscientist with a background in physics, biology and education research. Having worked in basic research since 2007, including experiments on rats and zebrafish, he is currently Lehrbeauftragter (temporary lecturer) at Tuebingen University. He is also a co-founder of Pro-Test Deutschland e.V., a non-profit encouraging transparency in animal research and improvements to animal welfare. Since 2017, he has conducted numerous communications workshops.

W2E1

What Is the Name of Your Strain?

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Abstract
Genetic quality control and microbiological quality control are at the heart of every animal care and use programme. However, it is evident from publications that the attention for the genetic quality is not reflected by the strain names reported in publications, presentations, etc. In research involving strains of mice, it is critically important to use the official name of the strain according to internationally accepted nomenclature rules. Using the right name will contribute to scientific accuracy and reproducibility, communication and collaboration between scientists and traceability of strains. The International Committee on Standardized Genetic Nomenclature for Mice and resources like the Mouse Genome Informatics (MGI) database provide guidelines and standardised names for mouse strains. These resources should be consulted to ensure compliance with nomenclature rules. In this workshop, the rules and different resources will be introduced, but the emphasis will be on working in groups on different assignments ranging from beginner to advanced. Target audiences are breeding coordinators, researchers, animal techs of breeding units, and veterinarians. Participants are expected to bring their own laptops with WIFI connection to be able to access web-resources.

W3A1

Promoting Accurate Statistical Reports on the Creation and Breeding of Genetically Altered Animals

B. Pintado[1], D. Anderson[2] and K. Mesbah[3]
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[2]*LASA, London, United Kingdom*
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Abstract
The European Union's commitment to transparency in the use of animals for scientific purposes requires the collection of accurate information by Member States. This information is provided in two ways: the annual report on uses, which pertains to animals specifically involved in procedures, and the 5-year Implementation report which includes information on animals bred but not used. Genetically altered animals (GAA) have characteristics which can make it difficult to ensure a consistent and correct classification in these reports.

This practical workshop is aimed at those individuals providing statistical data on GAA creation, breeding and use to the competent authorities. To achieve the desired training for a consistent and accurate data reporting, prior knowledge of basic concepts regarding GAA management, such as the creation, establishment, and maintenance of genetically altered lines, is necessary. The workshop intends to provide the necessary minimum knowledge to enable GA animals to be classified correctly in the different reporting categories. It is structured as a practical and interactive session in which a short introductory talk and a general quiz will be followed by splitting the attendees into different groups where common practical situations found often in any GAA facility will be presented. Each group will be asked to prepare a submission of the statistical data report based on the case material provided. These reports will then be presented and discussed collectively, also providing an opportunity to address any questions from the attendees.

W3B1

Self-made Score Sheet or Not? That's the Question

P. Bugnon[1] and M. Heimann[2]
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[2]*ETH Zürich, Zürich, Switzerland*

Abstract
A score sheet is a very powerful tool for assessing the impact of an experiment on animal welfare, as long as it incorporates certain principles that make it an effective tool. Otherwise, the use of a poorly designed score sheet is a waste of the researcher's time and, above all, fails to achieve the objective of a proper constraint assessment. Furthermore, apoorly designed score sheet can have considerable negative consequences for the animal.

In addition to this constraint assessment, the score sheet defines the interventions to be carried out to reduce the impact of these constraints. Score sheets help to detect a constraint as early as possible to be able to react properly to minimize this constraint. Therefore, the degree of severity of the experiment is kept as low as possible. Finally, score sheets help researchers to stick to predefined humane endpoints of an experiment.

In this interactive workshop we will demonstrate a step-by-step approach to create an optimized score sheet for a defined experiment. This leads to a documentation tool that ensures the objective evaluation of constrain by all involved persons and supports reproducible reporting or publication.

W3B2.1

ETPLAS Working Group on DOPS Development for Practical Skills Assessment – Introduction and Overview

R. Frias[1], A. Holmberg[2], A. Costa[3], D. Kylmann Hansen[4], I. Tiebosch[5], R. Vlasbom[6], L. Withfield[7], A.-D. Degryse[8] and J.-B. Prins[9]
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[9]*Leiden University Medical Centre, Leiden, Netherlands*

Abstract
The ETPLAS Working Group 4 was established to develop Direct Observation of Procedural Skills (DOPS) and standardize skill assessments for EU Modules 3.2, 6.2, 8, and 20–22, in line with Directive 2010/63/EU and the 2014 Education and Training Framework. The targeted modules focus on practical skills critical for Functions A, C, and D, including animal handling (3.2), minimally invasive procedures (8), humane killing (6.2), minor anesthesia (20), advanced anesthesia (21), and surgical principles (22). The group aimed to create clear and user-friendly documents to facilitate the implementation of DOPS across EU institutions and beyond. Initial work involved reviewing learning outcomes and developing assessment criteria, descriptors, and scales, primarily focusing on mice, the most commonly used species in scientific procedures. Regular group meetings facilitated collaboration and continued progress. Development in DOPS evolved through continuous refinements of drafts. Key milestones included finalizing rubrics for EU Modules 3.2, 6.2, 8, and 20–22, focusing on laboratory rodents but adaptable for other modules and species. The group has refined scoring methods, integrated professional behaviors into rubrics, incorporated feedback from pilot studies, and benchmarked against international standards, particularly those from medical education literature. The finalized DOPS templates will be accessible on the ETPLAS website (etplas.eu). Next steps include drafting a manuscript with detailed instructions, expanded guidance, and references. This initiative aims to promote ethical research, harmonized training and assessment practices, and improved mobility of skilled professionals across the EU.

W3B2.2

Understanding the ETPLAS DOPS

I. Tiebosch[1], R. Frias[2], A. Costa[3], A. Holmberg[4], D. Kylmann Hansen[5], R. Vlasblom[6], L. Whitfield[7], A.-D. Degryse[8] and J.-B. Prins[9]
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Abstract
For the purpose of harmonising the assessment of skills and competence described in the The education and training framework to fulfil the requirements under the Directive 2010/63/EU1, a working group of ETPLAS has developed Direct Observation of Practical Skills (DOPS). They are developed to be used for the assessment of practical skills related to the EU modules 3.2, 6.2, 8, 20, 21 and 22, and are developed in line with the online module on competence assessment to be published by ETPLAS (https://etplas.eu/). The assessment sheets will be presented during the workshop "Using the ETPLAS DOPS for assessing the practical skills of personnel working with laboratory animals". They consist of a four level rating scale to score proficiency 3 to 4 assessment criteria with scoring examples. The rating scale is then translated into global rating scales of practical competence and the level of supervision needed if training is to continue, and is concluded with feedback and remarks. This allows for hands-on practical assessment during training or supervision, to aid in the insurance that animals used in science are always properly handled and cared for. The workshop will support you with tools and tips for understanding and using these DOPs and for conducting assessment of competence in practical tasks.

W3B2.3

Practical application of the ETPLAS DOPS

L. Whitfield[1], I. Tiebosch[2], R. Frias[3], A. Holmberg[4], A.-D. Degryse[5], D. Hansen[6] and A. Costa[7]
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[4]*Karolinska Institutet, Solna, Sweden*
[5]*ETPLAS, Puylarens, France*
[6]*University of Copenhagen Department of Experimental Medicine, Copenhagen, Denmark*
[7]*i3S, Porto, Portugal*

Abstract
Do you train, or assess competence in practical tasks? Then this workshop is for you!

The practice of assessing trainees is a skill to be learned, in the same way that teachers and supervisors also require training, but unfortunately, assessor skill and training is often overlooked in the Lab Animal Sciences setting. This workshop will help everyone who evaluates competence to use the ETPLAS DOPS (Directly Observed Practical Skills) assessments, such as those relevant to modules: 3.2 (Species-specific Biology Skills), 6.2 (Humane Methods of Killing Skills), and 8 (Minimally Invasive Procedures without Anaesthesia Skills).

The workshop has an interactive, hands-on style and will explain how the DOPS sheets work, help you understand common issues that you may encounter during assessments and provide practical tools and tips to help. The atmosphere is friendly - we welcome experienced assessors and beginners equally, encourage you to have a go, and provide feedback on assessment process. Please also come ready with your questions about assessment and we will use the group discussions to find some solutions.

W3B2.4

Case Studies and Real-world Application

A. Costa[1], R. Frias[2], A. Holmberg[3], D. Hansen[4], I. Tiebosch[5], L. Whitfield[6], A.-D. Degryse[7] and J.-B. Prins[8]
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Abstract
Developing instruments/tools for competence assessment that ensure key measurement properties such as validity and reliability is challenging, time-consuming, and requires considerable effort. Furthermore, the administration of a well-designed assessment instrument is crucial for standardizing and harmonizing assessments, ensuring consistency and fairness across diverse evaluators and contexts. This was one of the key objectives of the ETPLAS working group when developing instruments for assessment.

Nevertheless, despite the group's efforts to create high-quality instruments, the success of their application in workplace-based assessments will always depend on factors that may limit or jeopardize their use. Examples of these factors include the multidimensional nature of competencies in LAS as the aim of assessment, the inherent limitations of the instruments, variability in contexts, the need to assess competence in executing techniques and procedures that are themselves subject to constant refinement and optimization, and the potential biases or insufficient preparation or training of assessors. Clear guidelines for training assessors and regular calibration sessions to minimize subjectivity

and variability are essential to enhance the effectiveness and reliability of these tools.

Focusing particularly on instruments designed for the LAS context, this workshop session invites participants to explore the various challenges that supervisors and assessors may commonly face when administering an assessment instrument to support the judgment of competence in practice.

W3B3

Score Sheets in Aquatics: Let's Swerve Between Species and Models

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Abstract
Score sheets are a mainstay of research reporting but few score sheets for aquatic animals are published in the literature. Assessment of the welfare of aquatic animals can be challenging, and scoresheets can really help collect data on adverse effects and harmonise severity assessment. Scientists use score sheets daily but often do not detail them in their publications. New tools are available too. This workshop hopes to highlight what is available, how to adapt and use score sheets, and share experience on the wide variety and context score sheets can be adapted to. Bring along your score sheet or discuss what we prepared for you!

The workshop will start with a deep dive into scoring killifish depending on their developmental stages and the models. We will then look into a system to collect scores for individual fish and their population. The third presentation will browse through score sheets used in a wide range of context, such as wild fish, Xenopus, as well as zebrafish undergoing heart cryo-injury. The three presentations will set the basis for the final part of the workshop where attendees will be asked to score fish and justify their score for various clinical signs. We will explain how the score helps to assess severity.

W3B3.2

Population Assessment- Let's Score Ageing Zebrafish in Relation to their Strain

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Abstract
Welfare assessments for genetically altered zebrafish strains throughout their life span including ageing effects are still in their infancy compared to rodents. In contrast to rodents, severity assessments in teleosts need to reflect the whole population and should ideally capture the time course since birth to death. The lack of guidelines and tools presents a challenge for researchers to justify severity assessments and refinement methods for genetically altered and ageing zebrafish in animal permits and in front of ethics committees. In this workshop, I will introduce an adaptable, user-friendly score sheet for fish. The parameters contained in the excel tool are based on a literature review, have been validated by expert interviews, and evaluated by a fish pathologist. The tool allows to score groups, calculates summary scores and visualizes trends. I will show my recent attempts to employ this tool to score ageing zebrafish in relation to their genetic alterations and talk about pitfalls, trendlines and differences between genetically altered strains.

W3D1

Tools for Those Involved in Training and Assessing Surgical Procedures in Laboratory Animals

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Abstract
There are several documents available that describe how to achieve aseptic survival surgery in laboratory animals, but the practical aspects of training usually rely on the experience or the preference of the trainer. In this sense, it is essential to create a supportive, structured, reproducible, fair and consistent system for surgical training and assessment, to allow flexibility across different fields of research and to incorporate the frequent changes that come with advances in surgical technique and welfare. Effective training is a crucial component not only to the career development of the trainee, but also to ensure a culture of care at the institution.

Direct Observation of Procedural Skills (DOPS) is an extensively used tool to demonstrate competence, and consists of a structured checklist comprising all steps that the trainee needs to complete during a specific procedure. To ensure consistency across assessments, standards should be clearly defined, describing what are the pass/fail criteria. Effective training and assessment also involve other aspects such as communicating effectively when a new refinement wants to be implemented and approaching issues of limited progression when learning new skills.

W3E1.1

Key Concepts in the Outputs from the FELASA Experimental Design Working Group

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Abstract
This talk will illustrate the key concepts underlying the outputs of the FELASA Experimental Design Working Group. These outputs include learning outcomes and a tested programme for experimental design courses, guidance on running such courses and suitable tutor skills.

One underlying concept is focussing tuition on covering key learning outcomes about clear experimental questions, randomisation and blinding, experimental units, basic hypothesis testing, simple comparisons, blocked designs and factorial approaches, and developing from these as time allows. Another is progression, establishing a good understanding of the basics before moving on to the more complex matters, and a third is bringing the practicalities of conducting an animal experiment into all the considerations, particularly how those affect bias, the experimental replicate and the choice of design.

An important concept is learning through doing as well as listening, so including in courses participant group discussion, problem solving and exercises which bring out the main issues. Illustrating this concept will provide an introduction to the subsequent group activity in which attendees can use and discuss some of the educational material developed.

W3E1.2

How Early Years Researchers Responded to These

M. Forni[1], M. Berdoy[2], N.H. Franco[3], D.J. Fry[4], C. O. Sorzano[5] and T. Steckler[6]
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Abstract
This talk will illustrate the role and the results of feedback obtained after the course organized by the FELASA Experimental Design Working Group (EDWG).

Courses tailored to young researchers and professionals must follow the principles defined for higher education, with particular emphasis on integrating theoretical foundations and practical applications to foster active learning and problem-solving skills. Ensuring the alignment of the course with best research practice is essential. Collecting participant feedback both immediately and after a delay is vital for evaluating and enhancing the programme's practical impact and effectiveness on the community. It is a key step in the implementation process, closing the Quality Cycle and ensuring the continuous improvement of educational quality.

Immediate feedback on the EDWG course usefully captured participants' real-time impressions, with the satisfaction rating of 4.5/5 giving confidence in the relevance of course content and the quality of instruction. It also picked up logistical issues. The late (one-year) feedback provided an indication of how participants had applied the training in real-world settings, particularly in their research and teaching practices. This long-term evaluation (satisfaction and usefulness rating 4.75/5 and 4.33/5 respectively) helps measure the practical effectiveness of the training, and inform future iterations of the programme.

Involving participants in both types of feedback promotes a more adaptive, student-centered approach, and has fostered ongoing mentorship, peer collaboration opportunities and a sense of inclusion. This approach encourages participants to take an active role in shaping experimental design education, ultimately benefiting the broader Laboratory Animal Science community.

W3E1.3

Double Trouble? Tackling Misgivings about Size of Experiments Including Both Sexes and/or Two Strains

N.H. Franco[1] and D.J. Fry[2]
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Abstract
The prevalence of single-sex studies in the preclinical literature is cause for concern. Unless there is good reason, such as study of the reproductive system, using just one sex risks misrepresenting half of the population being modelled, with ethical and clinical consequences. In discussion with researchers on this subject they often voice misgivings about the increased numbers of animals that will be needed in two-sex studies, in the beliefs that oestrous cycles in females lead to higher variability in research parameters or that using two sexes would require using twice as many animals. There is also concern that exploring effects in different strains involves doubling or trebling the number of animals used.

Effective education and training in experimental design and statistics should tackle these misgivings (1) by providing an understanding of why the underlying beliefs are mistaken. This talk will cover which experimental designs can accommodate the use of two sexes or two strains concomitantly with other factors being studied, and the proper sizing of such experiments (2). It will also

introduce the subsequent group activity, which will involve discussion of case-study examples that can be applied in experimental design teaching and sizing exercises (3). Use of such an approach should help achieve meaningful learning of key concepts, hopefully leading to sounder ethical research practices, and ultimately more reliable, and generalizable results.

W4A1

Practical Guidance for Implementation of an Institutional 3Rs Strategy

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Abstract
Background: To embrace an Openness and Transparency agenda and to have a commitment to an appropriate Culture of Care, it is essential to put in place an Institution-wide 3Rs strategy. This makes it clear that the 3Rs should be at the forefront of ALL research carried out at an institution, acknowledging that work in non-animal areas is as critical as that with animals.

A formal institutional 3Rs strategy demonstrates this commitment to the staff and students of the institute, funders and to the wider public. This also ensures that the Culture of Care Commitment on the 3Rs is embedded and consistently applied. As well as being a legal requirement, there is also a public and funders expectation that researchers and institutes will always fully apply the 3Rs. It is appropriate to embed a 3Rs strategy within institutional research governance structures and processes to ensure that the concept of the 3Rs is pro-actively, continuously, and consistently, embraced.

Workshop Outcomes: This workshop will provide practical guidance on implementation of an institutional 3Rs strategy. Attendees should be able to take the strategies presented and immediately apply those appropriate to their own institution.

Workshop structure: By using the approaches taken at two different institutions, and discussing what went well and what went badly, the workshop will provide practical guidance to attendees that they can tailor to their own situations. The workshop will integrate short presentations with interactive questions and activities.

W4A2

Mastering Non-technical Summaries (NTS) Writing: Enhancing Transparency and Ethical Accountability

N.M. Goncalves[1] and B. Tolliday[1]
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Abstract
The workshop aims to equip participants with skills to create effective non-technical summaries (NTS) that comply with legal requirements and enhance public understanding and transparency in animal research. NTS are crucial in EU legislation, best practices for writing and presenting them, and strategies for handling public inquiries and media interactions.

NTS bridge complex scientific research with the public, providing clear information about animal use in biomedical studies for diseases like cancer and dementia, and rare genetic disorders. They are required by law in the European Union and welcome by researchers and institutions to engage more openly with the public. However, NTS often contain complicated scientific terms and concepts and lack vital information for interested audiences.

NTS explain why animals are used, how researchers use the latest scientific methods to potentially replace or reduce their use, and how these methods minimize harm. They should include details about the species used, procedures, expected benefits, and potential harms.

The workshop goes beyond EU mandates, providing a comprehensive understanding of NTS's role in ethical accountability and transparency in animal research. Participants learn to communicate complex scientific information clearly and accessibly, ensuring NTS bridge researchers and the public.

W4B1.1

An Alternative Approach to Minimising Harms

C. Ramsden[1]
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Abstract
Whilst there is still a regulatory requirement to perform OECD 203 tests (Fish, acute toxicity) it is important that every single test is performed to the highest possible standard. Key pieces of information are needed before the commitment to the in-life phase of a test. The analytical method should be validated, sample storage stability should be proven and the behaviour of the test item in the test system should be understood prior to sourcing fish. Fish of the highest quality and health status should be used in the test. Tests should only be performed by experienced, licensed staff with thorough knowledge of the test type. Exposing fish to the test item should be the last step in the process. When the test is performed it should be known in advance whether the threshold approach and the use of the $LC^{50\ moribund}$ endpoint will be acceptable to the regulatory authority, and if there is any doubt the test should be performed as the full definitive version as specified in the test guideline with mortality as the endpoint. This is the most conservative approach which ensures that a regulatory authority cannot reject a study. While it is desirable to reduce the numbers of fish used in these tests and to limit their level of suffering during the test, it is of utmost importance to ensure that tests are not repeated due to poor performance of the laboratory or because the regulatory authority does not accept the approach used.

W4B1.2

Approaches to Refining the Fish Acute Toxicity Test

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Abstract
Animal testing is still an unavoidable need in the assessment of chemical hazards, especially for the environment which is tasked to avoid harm on all forms of life. Fish are recognised sentient (have the have the capacity to experience positive and negative feelings such as pleasure, joy, pain and distress that matter to the individual) species and a key ecological group. It is therefore not surprising that the Fush Acute Toxicity Test (OECD Test Guideline 203) is a standard requirement for chemical hazard assessment. What is not consistent with neither modern toxicology (i.e. Net Generation Risk Assessment; NGRA) nor ethical acceptance by the public, however, is the requirement for mortality as a regulatory endpoint. The origin of the requirement is discussed along with refinement approaches and progress made in the regulatory space following the most recent update of TG203 in 2018. This talk will also identify what remains to be done to improve fish welfare and minimise severity in regulatory hazard assessment

W4B1.3

Reducing Severe Suffering in Regulatory Toxicology Testing Using Fishes

J. Rodda[1], C. Stevens[1] and P. Hawkins[1]
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Abstract
Mortality is a key cause of severe suffering, and there can be an increased risk of this in tests using fishes, e.g. in regulatory toxicology procedures within OECD 203 and OECD 210 testing guidelines. In November 2023, the RSPCA organised an in-person meeting focusing on the application of humane endpoints in regulatory toxicology studies that use fishes, with the aim of identifying and sharing strategies to reduce and avoid severe suffering. The meeting also explored some of the challenges associated with this goal. Two of the main conclusions from the meeting centred on standardising approaches to (i) identifying sublethal clinical signs and applying humane endpoints, and (ii) improving staff training around identifying clinical signs, fish behaviour and welfare.

As a result of the meeting, the RSPCA has produced a report with specific recommendations for the wider scientific community, including regulators, scientists, animal technologists and unit managers, and training organisations. This talk will explore these recommendations and discuss how progress could be made towards reducing severe suffering in regulatory toxicology.

W4B2.1

The Regulatory Framework and Guidelines That Govern the Use of Live Animals in Training

N. Linklater[1] and A. Teubner[2]
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[2]Maastricht University, Maastricht, Netherlands

Abstract
The framework governing education and training for working with animals in research is based on Art 23 (2) Directive 2010/63/EU requirements in conjunction with Annex V. In 2014 an EU expert working group proposed a modular approach to gain specific key competencies that need to be obtained before people can start working with animals, which has been widely adopted. FELASA adjusted it's accreditation scheme to the modular approach. Even though the modular system helps to harmonize LAS education and training, differences in the set-up of courses and national requirements in the Member States still exist today, e.g. regarding the use of live animals for skills training and when a person is deemed competent to work unsupervised.

By the example of FELASA-accredited courses this talk aims to outline some key differences and commonalities. One take away will be that while a harmonized approach to education and training in LAS is an important refinement aspect, taking local requirements and tailoring courses to peoples training needs into account is essential to ensure appropriate training for different target groups.

W4B2.2

Use of Live Animals in FELASA-accredited Courses for Function A, C and D

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Abstract
LAS education and training is based on the requirements of art. 23 (2) Directive 2010/63 and Annex V of the directive. These requirements were translated by an Expert Working Group (EWG) into a modular approach to education and training in 2014 and updated in 2018. This approach aims at harmonization and standardization of LAS Training and Education across Europe and has been adopted by most non-/accredited courses.

Modules 3.2, 6.2 and 8 concentrate on the conveyance of practical skills (handling and fixation, euthanasia and minimal invasive techniques without anesthesia).

Against the background of the 3Rs, the text of the EWG can be and is interpreted differently with respect to the use of live animals in the aforementioned modules. Some countries and Course Organizers themselves have defined LAS training and education to be carried out with regard to practical skills in (some) specific ways with regard to their unique settings. By the example of FELASA-accredited courses this talk will outline some of these

distinctions but will also stress common grounds. It will depict how national requirements and local adaptions are vested and will give an outlook to a common definition of a minimum use of live animals in FELASA-accredited LAS Training Courses.

W4B3.1

Looking Back, Moving Forward: Reducing Severe Suffering Through Retrospective Assessment

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Abstract
Due to the nature of the project, the type of species used, or questions related to the likelihood of achieving the objectives of the project, the Directive introduced a possibility to carry out retrospective assessment (RA) on completed projects. These are compulsory for projects containing severe procedures and/or using non-human primates.

RA, carried out by competent authorities, relies on timely, accurate and detailed information on the project provided by the research team. The Three Rs – Replacement, Reduction, and Refinement – are at the heart of RA. Severe suffering is a key area to focus and RA could be an effective tool to identify new innovation for the benefit of further Refinement.

Publishing RA results is crucial for the wider dissemination of new Three Rs solutions. Member States were free to choose whether public non-technical project summaries would be updated with the results of retrospective assessment. Only 15 Member States publish the results of retrospective assessment in the ALURES NTS database. To obtain maximum benefits of the efforts made under RA, and capitalise on all findings, the remaining Member States are free, and encouraged, to use the database on a voluntary basis.

Let's make the work carried out count for the animals and the future!

W4B3.2

Maximizing In Vivo Research Outcomes: Retrospective Review for Improved Practice

E. Stringer[1]
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Abstract
Whilst Directive 2010/63 Article 39 mandates retrospective assessment of those projects involving severe protocols, at the University of Birmingham (UK) we have implemented a retrospective review process for **all** in vivo projects undertaken at the university under the Animals (Scientific Procedures) Act 1986. This process not only verifies whether the aims of the project were achieved, but also provides an opportunity to reflect on challenges and issues, and captures scientific and 3Rs-relevant achievements for broader dissemination.

Importantly, to maintain engagement the process must be streamlined to avoid becoming burdensome, while still ensuring meaningful outputs. This presentation will outline the University of Birmingham's approach, from data capture to review and downstream dissemination. The aim is to prompt thought, and provide simple, impactful solutions that can drive change using the information gained from retrospective reviews.

W4B4

Principles of Rodent Aseptic Surgery: A Practical Approach to Surgical Asepsis and Suturing Techniques

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[5]*AO Research Institute, Davos, Switzerland*
[6]*Royal College of Surgeons in Ireland, Dublin, Ireland*
[7]*BSRC "Alexander Fleming", Vari, Greece*
[8]*National and Kapodistrian University of Athens, Athens, Greece*

Abstract
This workshop is designed for researchers, research technicians, veterinary professionals, and students eager to develop a solid foundation and understanding of rodent aseptic techniques and basic surgical skills. The session offers a blend of theoretical knowledge and practical experience.

Lecture Session: Mastering the Fundamentals. The workshop kicks off with a short lecture covering the essential principles of aseptic surgery.

- Asepsis and Sterility: Understanding the critical concepts of asepsis and sterility to prevent contamination and ensure successful rodent surgical outcomes.
- Patient and surgeon preparation: Tips and tricks on how to prepare rodent patients for aseptic surgery, and best practices for surgeons to maintain sterility throughout the surgical procedures.
- Basic Suturing Techniques: Step-by-step introduction to the two basic and common suturing patterns: Simple continuous and simple interrupted.

Hands-On Session: Practical Skills Development. Following the lecture, participants will engage in a hands-on session.

Divided into smaller groups and led by experienced instructors. Attendees will observe live demonstrations and will practice under expert supervision patient sterile draping, and basic aseptic surgery using a cutting-edge 3D-printed mouse laparotomy simulator. This immersive experience is designed to reinforce the theoretical content while building confidence in performing the basic tasks for successful aseptic rodent surgeries.

By the end of the workshop, attendees will be equipped with the knowledge and basic skills necessary to optimize their surgical practices, contributing to reproducible research outcomes and animal welfare.

This workshop aims to provide high-quality surgical training while strongly adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement).

W4D1

Supporting Trainees in Practical Training with Animals: How to Manage Individual Difficulties?

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Abstract
Proper training before handling and performing procedures on animals is key for good animal welfare and is central to Culture of Care. To support inexperienced learners in acquiring the psychomotor skills that are required to do experiments with animals is a demanding task. The context of LAS courses presents particular challenges, with the majority of trainees handling living animals for the first time in group sessions of limited duration. Trainers' expectation is that trainees acquire basic skills (such as the ability to restrain an animal or give an injection) by receiving instruction and practicing with guidance and supervision. However, often lack of experience is not the only hurdle to overcome. Trainees may present difficulties that impact their learning and sometimes the learning environment. Through our teaching of a FELASA-accredited course, we have identified several common issues that repeatedly tend to occur in practical training scenarios. Based on this experience, we have designed the "Train the LAS Trainers" workshop, which provides a structured approach for discussing common challenges in teaching practical skills to do animal experiments, in order to facilitate the sharing of experience across countries and teaching contexts. We have previously tested the workshop where it proved helpful both on the individual (understanding that other trainers share similar challenges) and the group (developing a shared strategy) level. Supported by the use of vignettes and a group facilitation strategy, this workshop invites participants to reflect on the approaches and actions that should be taken to overcome these impasses.

W5B1

A Taster of Complex Surgical Models in Classification and Reporting of Severity of Procedures

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Abstract
Directive 2010/63/EU requires that experiments be designed to cause the least possible pain, suffering, distress or lasting harm to the animals used in procedures and requires that all procedures be assigned a severity classification at project submission. Moreover, recent requirements for non-technical summaries ask for the inclusion of a 'best estimate' of the number of animals in each severity category.

Lastly, in the annual Statistical reports, each individual animal use must be reported, based on the actual experienced severity. Severity Classification is also key in determining whether or not an animal can be re-used in other procedures.

The implementation of a Severity Classification process, is thus a big challenge. Having a very good understanding of the subject is paramount for properly evaluating research projects and for allowing implementation of refinement in procedures. Reporting of actual severity improves public transparency. Consistency of severity assignment across Member States is of the utmost importance to harmonise project licensing and reporting.

The European Commission has encouraged FELASA to perform Workshops on the Severity Framework, including classification and reporting of Procedures

This Workshop is intended **exclusively for those who have already attended the 'Basic' Severity Classification Workshops** held across the EU in the last few years. A similar format to that used in those Workshops will be adopted but new models will be presented. It will include challenges confronted with when dealing with complex surgical models in higher species, used in chronic drug metabolism and pharmacology studies.

W5B2

A Taster of Complex Neurobehavioural Rodent Models in Classification and Reporting of Severity

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[6]*LASA, London, United Kingdom*

Abstract
This workshop is intended to explore further some of the issues related to Severity Classification and its Reporting in the context of complex experimental procedures. In this specific workshop, the audience will address complex studies in mice and rat models taking into account the cumulative severity animals may experience. The examples to be discussed and challenged will be based on neuro-behavioural rodent models.

This workshop is intended **exclusively for those who have already attended the 'Basic' Severity Classification Workshop** held across EU in recent years. A similar format to that used in previous Workshops will be adopted, but with new examples, focused on a mouse EAE model for disease and a complex behavioural challenge model in rats. Less time will be allocated to the general principles of Severity classification and reporting, allowing ample time for discussion of problems raised by attendees.

Target Audience: People experienced in classification of severity of procedures in neuro-behavioural rodent models

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PA001

Improving Your Study Outcome by Using the Resource Blood in Mouse Experiments

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Abstract

Blood is a vital resource that reflects the health status of an organism and provides insights into the immune system's responses to diseases. The assessment of human white blood cell differentials through flow cytometry is a widely used diagnostic method, yet there is a significant knowledge gap regarding mouse blood counts, often leading to the discard of blood in animal experiments.

This project presents a simple and efficient protocol, relevant markers, and gating strategies for analyzing mouse blood using flow cytometry. We provide reference values from 3-month-old and 24-month-old male and female C57BL/6J mice. Our findings show that peripheral leukocyte fractions vary by age and sex. We also discuss influencing factors and limitations that should be noted when using this method. This study addresses the current gap in information on flow cytometric analysis of mouse blood, laying the groundwork for future research.

We propose two applications: the first entails analyzing complete blood counts with samples obtained through cardiac puncture at the end of an experiment, enhancing both quantity and quality of results. The second focuses on specific leukocyte subsets at various time points during an experiment using blood drawn from the facial vein. Analyzing blood from the same mouse at different time points improves study outcomes and reduces the need for additional lab mice. Both approaches adhere to the principles of the 3Rs (Replacement, Reduction, and Refinement).

PA002

Embrace Transparency! the Future of Animal Research Is Looking Bright, Thanks to Open Communication!

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Abstract

The 3R principles are fundamental to animal research today. In terms of a strong responsibility towards the animal, the 3Rs have been extended to include aspects of reproducibility, quality assurance and sustainability of research data. Communication about animal research has also changed significantly. In addition, the constructive culture of failure is receiving more and more attention when we talk about critical events in the housing, breeding and scientific use of laboratory animals. 1 Almost 10 years ago, our team launched the CIRS-LAS.org database. 2 Any type of critical incident or failed experiment is reported anonymously in the online database. Registered users can read and learn directly from reported cases in the user area. They can also provide constructive feedback on how to prevent the incident in the future.

The presentation describes the development of the database, which is used worldwide, and uses case studies to illustrate the significant impact that active participation in a constructive error culture can have on the welfare of our laboratory animals. In addition, realistic examples are used to show how an error culture can be gradually implemented in a research facility. The future of laboratory animal science is transparent and everyone can and should learn from mistakes.

PA003

Advancing Ethical Standards in Brazilian Animal Research: Transformations, Regulations, and Innovations Under CONCEA

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Abstract

Since 2008, animal research in Brazil has undergone significant transformations, driven by the establishment of the National Council for the Control of Animal Experimentation (CONCEA). The enactment of Law 11.794/2008, or the 'Arouca Law,' introduced mandatory Animal Ethics Committees (CEUAs) and placed a strong emphasis on the 3Rs principles—Replacement, Reduction, and Refinement—ensuring ethical standards in scientific activities.

CONCEA oversees 1,014 accredited institutions and approximately 2,500 registered facilities, forming a robust regulatory

framework. Recently, CONCEA published its first "Relatório de Uso de Animais em Ensino e Pesquisa," which covers the period from 2019 to 2023. This report offers statistics on the use of animals in research and education, allowing a better understanding of what researchers and teachers are doing with the animals, building public trust.

In 2023, CONCEA released the "Guia Brasileiro de Produção, Manutenção ou Utilização de Animais para Atividades de Ensino ou Pesquisa Científica," a comprehensive and detailed manual that outlines best practices for animal care, handling, and housing. This guide fosters the harmonization of experimental protocols and prioritizes animal welfare. In 2024, Resolution Normative No. 69 was published, establishing criteria for reallocating animals previously used in teaching and research to new scientific or educational projects. This aims to reduce animal employment, enhancing ethical accountability.

Furthermore, Brazil is committed to aligning with global ethical standards and advancing scientific research responsibly. By implementing these regulations, aligned with international policies, our country seeks to redefine animal research practices and guarantee a balance between scientific innovation and animal welfare.

PA004

Strategies for the Implementation of the 3Rs in Research and Development Institutions in Uruguay

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Abstract
The implementation of the 3Rs principles—Reduction, Refinement, and Replacement—in research and development (R&D) institutions in Uruguay has been guided by Law 18611, which regulates the ethical use of animals in scientific activities. The National Commission for Animal Experimentation (CNEA) plays a central role in overseeing compliance and promoting best practices in animal research.

Uruguay's approach to the 3Rs focuses on four key pillars: specialized training courses, a personal accreditation system, institutional registration, and regular institutional monitoring. Over the past 15 years, this program has achieved significant milestones. Currently, 32 institutions are registered, and more than 800 researchers have been accredited. Annual training courses, tailored to the ethical and technical requirements of working with various animal species, including rodents, farm animals, and livestock, are a cornerstone of this initiative.

This publicly funded program stands out as a successful model for promoting ethical and efficient animal use in research, combining regulatory oversight with capacity building. However, challenges remain, particularly the lack of official training programs for laboratory animal technicians and veterinarians specializing in laboratory animal science. Addressing these gaps will be essential to sustain and enhance Uruguay's leadership in the ethical application of the 3Rs.

By integrating regulatory, educational, and monitoring strategies, Uruguay provides a robust framework for ethical animal research while striving to meet international standards and address local needs.

PA005

Optimizing Laboratory Animal Breeding by Data-driven Management and Interaction with Animal Technicians

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Abstract
Anyone producing laboratory animals is morally committed to minimize the number of surplus animals. This ethical concern has gained public attention and now influences breeding approvals. However, research animal breeding is a complex process influenced by numerous factors, making it difficult to exactly balance supply and demand. A special challenge is minimizing the surplus animals in the case of small breeds with either a complex genetic or a harmful phenotype.

The Central Animal Facility at Hannover Medical School (MHH) has implemented a comprehensive approach to optimize breeding management. This strategy combines long-term data collection with the expertise of animal technicians to address the challenges of laboratory animal breeding. Twice annually, we distribute a detailed report offering an in-depth overview of animal numbers per breeding projects to the researchers.

Long term data recording enables to estimate the breeding power of strains, whereas well trained animal technicians are able to respond quickly on alterations of abiotic and biotic factors leading to unexpected alterations in productivity of strains. Moreover, animal technicians familiar with their strains know special demands of strains such as light intensity, sensitivity to noise, draught. In addition, a comprehensive collection of strain data allows not only a forward-looking calculation of breeding performance, but also a retrospective view of the use of the animals.

Nevertheless, effective breeding management requires close collaboration between animal technicians and researchers. This partnership is crucial to meet the specific needs of experimental groups while minimizing surplus animals.

PA006

Successful Rehoming of Rhesus Macaques Involved in Neurophysiology Research

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Abstract
Article 19 of EU directive 2010/63 states that '*Member States may allow animals used or intended to be used in procedures to be rehomed, or returned to a suitable habitat or husbandry system appropriate to the species, provided that the following conditions are met: (a) the state of health of the animal allows it; (b) there is no danger to public health, animal health or the environment; and (c) appropriate measures have been taken to safeguard the well-being of the animal*'. The option of rehoming or adoption has been included in the Belgian Royal Decree.

At KU Leuven, neurophysiology research is performed in rhesus macaques. All animals have a headpost and most of them have one or more craniotomies. At the end of an experimental project and if there is no need to obtain tissue samples which require killing of the animal, the Animal Welfare Body evaluates whether animals can be re-used in a neurophysiology experiment, in a non-neurophysiology experiment, in a non-recovery procedure or if animals can be rehomed. Animals can only qualify for rehoming if their general health is good, if they are social and could thus easily adapt to a new social group and if the size of the headpost has a good chance of successful wound healing after removal.

Since 2021, we have successfully rehomed 6 macaques to different zoos located in Europe. Headposts were removed and although this took some time, the wounds successfully healed. They could successfully be re-grouped in their new habitat.

PA007

The 3Rs, Essential in Teaching Activities at Oniris VetAgroBio Nantes, France

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Abstract
It must be acknowledged that there is a certain inertia in changing teaching practices in biology training courses that use living animals as models. At Oniris VetAgroBio, the veterinary school in Nantes, France, driven by regulatory changes and societal pressure, we have renovated teaching that historically used living animals in the disciplines of physiology, pharmacology, surgery and laboratory animal sciences. Thus, in the veterinary training course, we have developed the learning of pharmacology by developing workshops that no longer use living animals and allow knowledge to be acquired in a fun way (analysis of experimental methodology on articles, use of simulation software from the University of Strathclyde Glasgow, viewing and analysis of videos of fixed and volatile anesthesia on rats filmed on site). As part of the regulatory training (designer, applicator, experimental surgery) that we organize, we have developed the learning of the first gestures of gripping, restraint, administrations on inert models (stuffed animals, mannequins, artificial ears, artificial skins), in the form of workshops adapted to the species studied, in order to promote a progressive approach towards the use of the living animal. This entire approach allows us to replace and drastically reduce the number of animals used in teaching. Refinement is also implemented by learning the first invasive procedures (blood samples from peripheral veins for example) on anesthetized animals. In summary, learners appreciate the effort made for the benefit of the 3Rs and recognize the effectiveness of the new teaching methods implemented.

PA008

How to Respond to an Outbreak: Effective Room Decontamination While Maximizing Animal Preservation

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Abstract
Laboratory animals in biomedical research are usually maintained under a Specific-Pathogen-Free (SPF) status, defined by a list of infectious agents excluded as potentially interfering with the research. The detection of an interfering agent belonging to the SPF exclusion list in a microbiological unit is commonly referred to as contamination. When a contamination occurs, it requires the complete elimination of carriage of this agent by the animals and consequently mass euthanasia of the animals housed in this microbiological unit. This represents a major animal welfare event for both the animals and the caretakers. This case study proposes to demonstrate how we handled the contamination of an infectious agent carried exclusively by mice (Mouse Parvovirus - MPV) at our large-scale breeding facility. The open-cage rat and mouse breeding area was decontaminated while remaining in operation, thus avoiding the meaningless euthanasia of all the rats present in the shared microbiological unit. This achievement required the strong conviction and commitment of the entire team (caretakers, veterinarians, and top management). This unique sanitary and animal welfare success was made possible thanks to the innovative spirit of the biosecurity and veterinary team and their proved expertise. This was the first occurrence to our knowledge that an open cage area at a commercial breeder has been successfully proven decontaminated without complete depopulation.

PA009

Into the Wild Infectious Load: Rodent SPF Agents Prevalence in Wild Mammals

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Abstract
Specific Pathogen Free (SPF) status for rodents used in biomedical research is critical to ensure reproducibility and is maintained

thanks to biosecurity to prevent animal carriage of interferent agents. When it comes to controlling contamination risks, we often speak about pest control, assuming that wildlife is a major reservoir of infectious agents of our laboratory rodents. However, few studies exist on the prevalence of these agents in wildlife to better understand the infectious load in wild mammals. Our study summarizes the results of pathogen screening by molecular diagnostic methods of wild mammals captured at Charles River facilities in Europe. Between 2015 and 2024, a total of 88 mammals were screened (56% of mice, 30% of rats and 24% of other rodents and pest mammals). Detected pathogens with highest prevalence were Helicobacter genus, Rodentibacter heylii and ecto- and endoparasites. Murine viruses were not frequently detected compared to rat viruses with high detection of Rat Parvoviruses (RPV and RTV) in wild rats captured. No agent except Helicobacter genus has been found in trapped shrews, suggesting that specific PCR probes for shrews could be beneficial for a comprehensive overview of infectious agent carriage in this species. The knowledge of these prevalences confirmed that wild rodents are a major source of infectious agents for our SPF colonies, putting pest control at the core of biosecurity. These local data led us to adapt our biosecurity risk assessment with modification of the frequency of health monitoring for prevalent agents and of our sterilization processes.

PA010

Teaching Animal Ethics to a Multidisciplinary Academic Audience

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Abstract
Bridging the communication gap between animal activists and biomedical scientists has been a central focus of my role as designated veterinarian for the laboratory animal ethics module in the Master's program "Animal Welfare, Ethics, and the Law" in Greece. Since 2020, over 90 students, from diverse academic backgrounds (philosophy, law, literature, veterinary medicine, theology, biology, pedagogy, finance, nursing, law enforcement, etc.) have completed this 12-week module, with more than half identifying as animal activists.

The module addresses law, ethics, and welfare practices in animal research, taught by five experts. Assessment includes a written exam and group essay. The latter simulates protocol evaluation committees based on Directive 2010/63, and students justify the pass/fail of simplified research protocols. Following course completion, graduates are invited to participate in an anonymous survey, assessing the module's impact on their knowledge, skills, and attitudes towards animal research. In this survey, 15% participation was noted.

The survey results showed that students expressed vivid curiosity and passionate opposition to animal research followed by constructive dialogue in class on topics such as the 3Rs (replacement, reduction, and refinement), severity assessment, alternative methods, and harm/benefit analysis. A key takeaway was the recognition that being a well-trained expert in animal research is essential for effective participation in protocol evaluation committees. Students appreciated the insights gained on alternative methods and reported psychological strain due to their anti-speciesism mentality when assessing animal research.

Listening to my students has enhanced my ability to communicate the complexities of animal research.

PA011

Patient Discovery: Fostering Ethical Awareness and Openness in Animal Research

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Abstract
As EU institutions make significant strides in openly communicating about animal research, engaging diverse societal audiences becomes increasingly important. Patients, direct beneficiaries of advancements rooted in animal research, are a key group frequently left out of these discussions. Engaging patients fosters transparency while empowering them as advocates for lifechanging/lifesaving research that still requires the use of animals.

It is rare for biomedical researchers to be in direct contact with patients and the knowledge they can provide. Patients frequently highlight overlooked symptoms or challenges stemming from chronic conditions, providing researchers with valuable insights that can refine study designs and improve the alignment of research with patient needs.

To bridge these gaps, the European Animal Research Association (EARA) has created the Patient Discovery project, which connects biomedical researchers with patients and caregivers, promoting mutual understanding and ethical awareness. Through guided tours of animal facilities, tailored presentations, and open discussions, participants explore the ethical dimension of animal experimentation in research, while seeing first-hand how important animal welfare is to researchers and institutions and how the 3Rs are prioritised. Fostering these direct connections between researchers and patients not only promotes transparency but also inspires purposeful advancements in biomedical research to directly address patient priorities.

This session will showcase the growth and impact of the Patient Discovery initiative that has expanded across several European countries, such as the Netherlands, Portugal and Switzerland, aiming to inspire research institutions to open their doors to the people who need biomedicine and animal research the most: the patients.

PA012

A Survey on Perceptions on Using Animal and Non-animal Models in Biomedical Research

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Abstract
The European Citizens' Initiative "Save Cruelty Free Cosmetics - Commit to a Europe Without Animal Testing" closed in August 2022 with 1,2 million signatures collected. The third point of the Initiative called for modernizing science in the EU, by committing to a legislative proposal plotting a roadmap to phase-out all animal testing.

After public hearing at the European Parliament, the Commission responded to the initiative in July 2023. The response expressed a strong support to the development of alternative approaches with appropriate funding and initiating a series of actions to accelerate the reduction of animal testing in research, education, and training.

Along the process, several organizations and societies published statements about using animals for scientific purposes.

Motivated by polarized debates, we conducted an international survey (in May-June 2023, collecting 199 responses), on the current perceptions in the field. Interestingly, 36% of respondents were unaware of ARRIVE guidelines, 41% were unaware of PREPARE checklist, 41% were unaware of pre-registration, and 35% were not familiar with the concept of Culture of Care. However, >75% agreed that progress in biomedical research depends on animal models.

At the same time, 63% of respondents agreed that faster transition to non-animal methods is warranted, whereas 46% agreed that there is sufficient and easily findable information available for non-animal methods. Among respondents, 30% used primarily non-animal methods, and 24% used both animal and non-animal methods in their research.

The results of the survey highlight the continued need for training, education and dissemination of relevant information.

PA013

Impact of Bedding Type on Sentinel-free Soiled Bedding Animal Health Monitoring

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Abstract
As a cornerstone of the 3R's principles, *reduction* should be directly applied to the science of animal health monitoring. Previous research has recently demonstrated the efficacy of agitated Sentinel-Free Soiled Bedding (SFSB) cages to monitor rodent colony health versus traditional sentinel animal monitoring strategies. SFSB is a PCR-based testing method where a matrix or material is used to capture dust and nucleic acids directly from soiled bedding, eliminating the need for sentinel animals. However, most studies utilized corn-cob bedding or a single bedding type. Here we evaluated the utility of additional beddings (multiple paper-based beddings, aspen chip and combination beddings) for use with SFSB strategies. We performed six experiments where high binding capacity matrices (n = 5) were placed in soiled bedding collected from infected donor mice (n = 5 per group) at 2-week cage change intervals. Donor mice were tested via PCR on arrival. After exposure to two weeks of soiled bedding, matrices were tested by PCR for rodent viral, bacterial, and parasitic infectious agents. We compared prevalence in each animal group to pathogen recovery on exposed matrices. Direct comparison between bedding types was not applicable as each experiment was individually performed. We found that all expected viral, bacterial, and parasitic infectious agents were consistently detected by SFSB replicates regardless of bedding type used. These data demonstrate that SFSB provides a reliable method to monitor colony health regardless of bedding type used. These novel findings provide support for the utilization of SFSB-based rodent health monitoring on a wider variety of bedding types.

PA014

Critical Gaps in the Ethical Review of Animal Research in Sweden

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Abstract
The Directive 2010/63/EU specifies that all animal research projects require ethical approval by a competent authority before animal use may take place. The ethical review must contain a harm-benefit analysis (HBA) whereby the total harm inflicted on the animals is weighed against the predicted benefit of the project. It must also ensure project compliance with the concept of 3R.

This study examined the regulatory requirements on applying researchers concerning the ethical review of animal research in Sweden, and to what extent submitted applications fulfil these requirements.

In total, 44 applications from 2020 (approx. 10% of all submitted that year) were thoroughly reviewed. It was found that information about harm, benefit and the 3Rs was often insufficient or occasionally altogether missing despite being central to an ethical evaluation. This risks hindering both the Animal Ethics Committees' (AECs') performance of a HBA, and assessment of how the 3Rs have been considered in the project.

Our results emphasize critical areas of concern, including regulatory ambiguities and frequent omission of essential information in submitted applications. To facilitate a thorough ethical review, detailed, relevant, complete and accurate information need to be provided by the applicant. We present nine action points to facilitate and improve the ethical review process, for the sake of both applicants and the evaluators (AEC). Besides improved welfare of the animals used in research, this approach will also safeguard research quality and impartiality, and promote transparency and public trust in the ethical review process as a whole.

PA015

The Marseille Declaration – Together We Prioritize Animal Welfare

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Abstract
Globally, the standards for animal care in research facilities vary significantly, influenced by differing perspectives on animal welfare. These differences are also reflected in international regulations, such as those of the European Union and the US ILAR Guides. The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International carefully assesses and advises Animal Care Programs based on local standards.

Despite these differences, one (bio)logical fact has emerged: only a healthy organism can serve as a reliable basis for meaningful scientific data. The WHO defines health as a state of complete physical, social, and mental well-being. If we aim to use data from laboratory animals as a reliable pilot for human studies, this principle must apply universally. Currently, no globally applicable standard outlines the conditions necessary to meet species-specific health requirements. Significant variations in the health of laboratory animals and the associated comorbidities due to stress and inadequate housing not only result in avoidable suffering but may also jeopardize drug development and patient safety.

In response, several pharmaceutical companies convened in 2022 under the motto, "We are not competitors when it is about improving animal welfare standards globally." The FELASA conference was the origin of the Marseille Declaration, which emphasizes animal welfare and health and invites collaboration. To date, 11 companies have signed the declaration, forming a coalition to exchange ideas and discuss necessary investments. This presentation will update achievements and the value that joining the Marseille Declaration Coalition offers to the pharmaceutical, chemical, and contract research sectors.

PA016

Board Game "Bioethics Commission' Meeting": A New Educational Approach for the 3Rs' Dissemination

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Abstract
The ethical review of animal experiments is a developing area in Russia. Various scientific institutions set up the Bioethics Commissions, however, its members and researchers do not always clearly understand the ethical review process. For the better understanding Rus-LASA representatives conducted the board educational game "Bioethics Commission' Meeting".

The aim of the game is the discussion of the project of an animal experiment by the teams of Bioethics Commission and Scientists. All players are randomly assigned the roles of those teams. The set of the game includes the cards with the items of the resources needed for the experiment; a playing field, on which the resources are placed and the game currency ("LAScoins"), the amount of which is determined by a die roll. The playing field possesses sections the "Adverse Effects" and "Measures to reduce pain/distress/suffering", which the Scientists must complete in a situation of insufficient resources. The task of the team of Scientists is to fill in the playing field so that suffering of animals in the planned experiment is minimized. The Bioethics Commission members construct an "ideal" experiment. During the Bioethics Commission' Meeting (the final round of the game), the 3Rs for this animal experiment are discussed, and finally the Commission may either accept or reject this project.

The game is intended to promote a 'culture of care' and disseminate the 3Rs; its introduction may facilitate communication between researchers and the Bioethics Commissions. The game has been repeatedly approved at the Rus-LASA' Conferences and training courses.

PA017

From Historical Data to Future Insights: New Approach for Analyzing and Re-Using Behavioral Datasets

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Abstract
Video tracking and computer-assisted behavioral analysis provide objective and consistent data, playing a crucial role in mitigating research biases and influencing factors. Driven by the need for transparency and reproducibility, these systems align with FAIR principles, promoting data sharing, reusability, and improved study design.

Over 15 years of behavioral experiments conducted in our laboratory on rodent models of cognitive decline—including Tg2576 and Ts65dn—have generated extensive datasets to phenotype genetically modified mouse lines and evaluate the impact of central nervous system-targeted therapies. Traditional protocols based on power analysis for sample size calculation often require large animal numbers and extended timelines, potentially impacting both animal welfare and data reliability.

Automated tracking systems provide tens of quantitative parameters during a single test, but statistical analyses are usually limited to one-to-one comparisons. We performed an in-depth re-analysis of our historical data, including all parameters from Y-Maze experiments, to identify potential combinations that could reveal earlier signs of cognitive decline in mice, also considering natural aging processes in wild-type controls.

Following ALCOA and FAIR principles, metadata were validated, enabling robust retrospective analysis to harmonize data, assess inter-assay variability, and correlate cognitive results. By revisiting all available dataset parameters, we uncovered opportunities to maximize the utility of existing data. Leveraging these datasets might provide better strategies for study design and sample size calculation, supporting the 3Rs principles by reducing the number of animals required. Additionally, it could foster laboratory organization for improved monitoring of inter-experiment variability.

PA018

Qualitative Exploration of the Perceptions of the 3Rs by Swiss Researchers: A Dual Loyalty?

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Abstract
The principles of 3R (Replacement, Reduction, Refinement) serve as a cornerstone for the current debates on the use of animals in research, aiming to minimize harms while advancing scientific discovery. In the EU and Switzerland, researchers are legally bound to comply with these principles and to include them into their research practice. Although numerous guidelines on the practical implementation of the 3Rs in research exist, only few studies explore how researchers perceive these principles in relation to their research. We conducted 37 semi-structured interviews with Swiss researchers (18 doing animal experimentation and 19 researching on 3R methods or non-animal methods) and analyzed them using reflexive thematic analysis. One key preliminary result is that researchers face unique challenges in implementing the 3Rs due to their dual loyalty – to the pursuit of sound scientific knowledge and ethical research practices. Based on our results, this dual loyalty took on four dimensions: (1) the loyalty to one's research and good science; (2) the loyalty to one's research and personal ethics; (3) the loyalty to one's research and community standards; (4) the loyalty to one's research and openness to the public. Our findings will contribute to a deeper understanding of the ethical and practical complexities in the implementation of the 3Rs by researchers, offering new perspectives for policymakers, funding bodies, and research institutions to support scientific innovation while meeting high research ethics standards.

PA019

The Combination of Immunodeficient Mice and Quail Chorioallantoic Membrane Model in Colorectal Cancer Research

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Abstract
With respect to ethical aspects associated with the use of animals in biomedical research, immunodeficient mice represent an irreplaceable tool in the derivation of patient-derived xenografts (PDX) used for searching for new treatment options or biomarkers.

Thanks to effective collaboration with clinics, we have processed more than 650 vital human colorectal cancer specimens, including liver metastases. Specimens were subcutaneously administered to mice in the presence of extracellular matrix. We observed higher efficacy of engraftment of a piece of tissue (to 10mm3) compared to administration specimens processed to single cell suspension. Application of unprocessed specimens is significantly less time-consuming and cheaper. Tissue is implanted under inhalation anaesthesia and oral analgesia. Mice are recovered after application, and no signs of pain or discomfort are observed. NSG mice are superior to SCID/bg mice for engraftment of metastatic tissue. Moreover, NSG mice are not predisposed to a lymphoma of the thymus. For PDX induction, we use exclusively animals bred in our facility. NSG mothers provide big litter, and offspring are habituated to animal keepers. Continuous production of defined numbers of animals enables their constant accessibility in a specified age and is economically favourable.

We replace the part of the experiments on mice with studies performed on the quail chorioallantoic membrane (CAM) model. CAM offers ethical and technical advantages; experiments take less time and money. The combination of in vivo and ex ovo approaches represents an effective and animal-friendly methodology.

The work was supported by the Slovak Research and Development Agency under the contract APVV-21-0296.

PA020

Collaborative Governance for Ethical Animal Research: Bridging Gaps Between Government, Institutions, and NGOs

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Abstract
Maintaining high standards in laboratory animal care and use is essential for ethical and effective preclinical research. We aimed to explore how such standards are upheld, emphasizing the collaborative roles of governments, institutions, and LAS-like non-government organizations in ensuring compliance and consistency.

Government policies establish foundational regulations for animal welfare, balancing ethical treatment with scientific rigor to ensure reliable outcomes. Where formal governmental frameworks are lacking, institutions and NGOs must strengthen their efforts to address gaps. National and international regulatory bodies provide oversight structures, enabling enforcement and continuous improvement of practices.

NGOs contribute significantly by advocating for animal welfare, advancing alternatives to animal testing, and monitoring compliance. Their role as watchdogs promotes transparency and accountability in research. Collaborations with institutions and public engagement initiatives help sustain societal trust and uphold ethical integrity.

Institutions supplement governmental roles by enhancing internal governance. Robust Insititutional Ethic Committees are vital for rigorous review and approval processes. Adopting globally recognized guidelines helps institutions standardize animal welfare and research quality, even in regulatory voids. Training and education further equip researchers with ethical competencies, fostering a culture of accountability.

A collaborative approach among stakeholders is critical to advancing animal care standards. By combining institutional leadership with NGO-driven advocacy, ethical research practices can thrive, even without formal governmental mandates. This multi-stakeholder synergy highlights the importance of shared responsibility in bridging regulatory gaps, ensuring that scientific progress aligns with ethical imperatives. Together, these efforts reinforce a global commitment to humane and credible preclinical research.

PA021

Effects of Sex Differences in General Anesthesia

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Abstract
The sex of an animal significantly influences its anatomy and physiology. There has been ongoing debate regarding whether male and female animals respond differently to anesthesia and whether it will have a substantial impact on daily practices. This presentation will provide a concise overview of selected anatomical and physiological aspects of mice and the role of various anesthetics in shaping the animal's anesthetic experience. This is an emerging field of research that lacks comprehensive understanding across the diverse range of inhalant and injectable anesthetic options. The presentation will also encompass appropriate anesthesia and analgesia techniques. The intended audience includes researchers, veterinarians, and members of AEC/IACUC/OB committees.

PA022

Long-acting Analgesia (Rodents) – Bench to Bedside

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Abstract
Long-acting buprenorphine, a commercially available medication, has been extensively utilized in research settings, particularly involving rodents. Despite numerous publications on this topic, the author will share his personal experiences from laboratory research to clinical practice. The presentation will showcase data on plasma concentration and behavioral analgesia. While the focus will primarily be on long-acting buprenorphine (e.g., buprenorphine SR, Ethiqa), the advantages of incorporating other long-acting analgesics, such as transdermal buprenorphine (Zorbium), will also be discussed.

PA023

Promoting Global Ethical Standards and Cultural Competency in Animal Research

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Abstract
This presentation provides an in-depth exploration of the essential principles needed to navigate the complexities of international animal research collaborations while maintaining the highest standards of ethical conduct and animal welfare. It begins with an overview of global guidelines and regulations, reinforcing the importance of adhering to ethical frameworks and upholding welfare standards across all research activities. Attendees will gain insights into how cultural competency plays a pivotal role in effective communication, fostering collaboration, and minimizing

misunderstandings in diverse research settings. The presentation also emphasizes the "culture of care" and its critical impact on fostering respect for the cultural norms and practices of international collaborators.

A key focus will be placed on accountability in research practices, highlighting strategies for transparent communication, accurate reporting, and team integrity to build trust within international partnerships. Methods for establishing and maintaining productive global research collaborations will be shared, including approaches for conflict resolution to address and resolve cultural differences. Ethical decision-making will be discussed using real-world scenarios to illustrate the importance of culturally sensitive approaches to ethical dilemmas.

The presentation will also provide practical guidance on how attendees can translate these concepts into actionable steps within their own research environments. Resources for ongoing learning and support will be shared to encourage continuous development in cultural awareness and ethical practices. The session will conclude with a call to action for attendees to embrace reflection, feedback, and collaboration as key drivers for sustaining impactful global research partnerships.

PA024

Using Species Selection to Reduce the Severity in Animal Research–A Qualitative Interview Study

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Abstract

One measure to reduce the harm inflicted on animals during a research project is the selection of species for the experiment. This process is a legal requirement in some European countries. The German animal welfare law, for example, demands the choice of the animal that would suffer least from the specific circumstances of the experiment; the Swiss one the choice of the species that is lowest on the evolutionary scale.

Both pieces of legislation present demanding tasks for decision-makers in animal research. Not only do they require exact information on all animal species that might be suitable for the experimental purpose. They also have to weigh this ethical consideration against many other ethical but also pragmatic, logistic, or bureaucratic factors that come into play when designing a study.

In ca. 30 qualitative, semi-structured interviews on animal research regulation with animal research stakeholders from Germany and Switzerland, we addressed the challenging task of animal model selection. After a reflexive thematic analysis after Braun and Clarke (2016), we compared the identified themes and sub-themes with factors for animal model selection presented by the literature (Dietrich et al. 2020).

While in theory the process and the justification are demanding, the actual animal model selection among our field of participants is often shaped by institutional and logistic, habitual, financial or publication-related constraints and scientific standards, but also by personal boundaries of the researchers. Additionally, the participants point towards a lack of both, information on species-specific capacities and guidance in the species selection process.

PA025

Systematic Review Reduces False Positive Interpretations of Animal Experiments Data

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Abstract

One of the reasons of the poor reproducibility in pathology modeling is the bias in interpretation - researchers tend to overestimate data confirming the success of model development. Our study is devoted to the quantitative measurement of this bias. We assessed the effect of bias in working with master's students. During the course, they had to reproduce one of the simple models in mice and to draw reports with conclusions about the success of model development. In this case, the students pre-prepared a systematic review for one of the models, i.e. one of the models was familiar to them, and the others were not. A total of 13 students participated in the study, each of whom worked with two animal models. Before analyzing the reports, an independent reviewer eliminated errors caused by data handling or statistical analysis, i.e., left only misinterpretation errors. As a result of the subsequent analysis, we found that preliminary writing of a systematic review significantly reduces the frequency of misinterpretation. At the same time, the frequency of false-negative interpretations ("The model does not work", when it actually worked) did not differ between students familiar and unfamiliar with the model, while the frequency of false-positive interpretations ("The model works", when it actually did not work) was 4 times higher among students unfamiliar with the model. We showed that the introduction of mandatory writing of a systematic review into research practice significantly reduces the frequency of false-positive interpretations of the results of animal experiments.

PA026

Ethical Improvement of Efficacy Study of a New Treatment Using GOTTINGEN Minipigs

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Abstract
Developing a swine model is a pivotal step in preclinical research to assess the efficacy of treatments, devices, or therapies. This approach leverages the anatomical and physiological similarities between pigs and humans, particularly in cardiovascular and skin systems, making it highly relevant for translational studies. Our reference model is the anesthetized domestic swine monitored over 6 hours.

We recently performed an efficacy study to obtain marketing authorization for one promising treatment, as required by the French National Agency for the Safety of Medicines, which led us to reassess our model.

To gather more data using fewer animals, we decided to work with awake animals and extend the observation period from 6 h to 7 days. A comprehensive telemetry monitoring system was implemented to support this new approach. Moreover, to ensure homogeneous and comparable results, the porcine breed had to be identical to the one employed by our private partner performing the preclinical toxicology study, the Göttingen minipigs. We, therefore, adapted our protocol from the domestic swine to the minipig.

We established optimized protocols combining improved animal welfare and enhanced care practices for the minipigs. We completed specific surgical training on implanting telemetry transmitters under the guidance of Delphine Bouard (Vetsalius®). A collaborator from the University of Lille (UFR3S) trained us for daily blood sampling without anesthesia or stress, ensuring minimal adverse effects on animals.

In conclusion, reassessing our standard swine model for efficacy studies proved to be highly beneficial ethically, paving the way for more humane and scientifically rigorous practices.

PA027

PubCompare: Enhancing Protocol Search, Comparison, and Reproducibility in Preclinical Research

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Abstract
Artificial Intelligence (AI) tools are transforming the development of scientific protocols and literature reviews by automating routine and time-consuming tasks, particularly in identifying methodologies critical to preclinical research. Ensuring reproducibility is essential, as misinterpretations and a lack of transparency in methods can compromise research validity. Failure to replicate published findings raises concerns about the reliability of preclinical studies and introduces ethical questions regarding the continued use of animals.

We aimed to validate the PubCompare tool academically, focusing on its application to finding and comparing laboratory animal science protocols essential for preclinical research. Testing involved widely used protocols in rodent models of anxiety and depression, including the Open Field Test (OFT), Sucrose Preference Test (SPT), and Chronic Unpredictable Mild Stress (CUMS). Preliminary results showed PubCompare achieved high accuracy for OFT (90% correct across five observers, including students and researchers) but lower accuracy for SPT (68%) and CUMS (80%), particularly for complex protocols.

We also evaluated the time required to find the exact information using traditional methods, discovering that manual processes were significantly slower. Testers valued PubCompare's ability to locate and extract key protocol information and its functionality to present studies side by side, highlighting methodological differences. PubCompare's database, containing open-access and paywalled studies, reflects common challenges in research accessibility. Despite its utility, our findings suggest that PubCompare, as a new platform, requires refinements to enhance usability, robustness, and ability to process complex methodologies. Addressing these areas will make PubCompare a more effective tool for supporting reproducibility and advancing preclinical research.

PA028

Integrating Artificial Intelligence in Ethical Evaluation: A Model for Brazil's IACUCs/CEUAs

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Abstract
We are developing a software tool to assist the Brazilian Ethics Committees on Animal Use (CEUA) in reviewing and approving animal experimentation projects, and the tool was based on national legislation (CONCEA's guidelines), international guidelines, and decision theory. The tool generates a numerical value for each project, according to the risk identified in the project, and it also provides severity scores and guidelines for improving the projects. The tool is based on the 3Rs to ensure that the experiment is approached correctly to the animals and science, and it can review the project in real-time, providing feedback to researchers and CEUAs with possible changes and actions to comply with the legislation. Therefore, this should facilitate the process of analysis of the projects and stimulate researchers to increase their efforts to implement the 3Rs. We are validating the tool, and the results so far were satisfactory, so it identified areas of concern and provided recommendations consistent with expert opinion 85 percent of the time. It also can save CEUA members hours of reviewing these documents, allowing them to focus on their priorities. Thus, the software also has the potential to educate scientists because it identifies problems and suggests ways to

address them. This software represents a significant shift in the way CEUA members work, and it provides them with a tool to ensure the research is ethical, promotes science, and protects the animals. Therefore, it is an innovative use of technology to do the right thing.

PA029

Analysis of Published Non-technical Project Summaries in Austria in 2021 to Assess Replacement Methods

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Abstract
Non-technical project summaries (NTPS) are easy understandable summaries of research projects involving animals. They are accessible for everyone and should inform the general public about approved animal experiments. Besides information about objectives and the potential benefit of the study, they must include considerations regarding the 3Rs. Since January 1st, 2021, NTPS are published in the EU database Animal use reporting EU System (ALURES).

To assess which replacement methods are already taken into account and to identify gaps in replacement methods, NTPS were extracted from ALURES.

After screening the database, 126 NTPS from Austria from the year 2021 were included in this pilot study. The following sections of NTPS have been reviewed: species, planned number of animals, estimated severity level, survival, target group, purpose(s) of the project, aim of the animal experiment. Additionally, information regarding replacement was extracted. The sections target group, purpose(s) (equates to special field) and aim of the project as well as type of replacement and reasons for absent replacement were defined and categorized for further quantitative analysis.

Our results showed that most studies are conducted in the field of "basic research" and aim to benefit "patients". One third of the project leaders mentioned employing non-animal methods within their projects, whereas cell culture, human stem cells and embryos are the most common methods. The most common answer for denied replacement was "lack of systemic and cellular complexity".

Further analysis to assess trends in the use of alternatives in Austria over the years are planned.

PA030

Reducing our Reliance on Animals through Advanced Technologies: Today and Tomorrow

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Abstract
Successful preclinical pipelines rely on the models' ability to predict the safety and efficacy of drug candidates in patients. Advanced technologies such as complex multimodal imaging, OMICs, New Approach Methodologies (NAMs) and AI/ML can complement traditional in vivo and in vitro models, maximising data insight from each study, refining models and accelerating decision-making. This presentation outlines examples of such complementarity where advanced technology helps bridge the cross-species translational gap and reduce our reliance on animals.

Imaging technologies have become a key aspect of pharmaceutical R&D, helping to understand the complexity of tissue biological events. Spatial-omics gives access to transcriptomic, proteomic and metabolomic data in a single tissue slice. AstraZeneca integrates those data with gold-standard histological methods to generate a holistic understanding of drug action using Mass Spectrometry Imaging (MSI) and Imaging Mass Cytometry (IMC). Integrating both technologies is pivotal in providing pharmacokinetics, pharmacodynamics, safety and target engagement information, which is crucial for decision-making.

NAMs are human-derived in vitro or in silico models developed as primary proxies for human biology. Examples of complex, self-organising cellular models, such as organoids or microphysiological systems (MPS), will illustrate how improved predictivity helps implement the 3Rs and reduce our reliance on animal use.

With the rise of NAMs, reducing or replacing animal byproducts (e.g. FBS) in cell culture is required for ethical and scientific reasons. Preliminary results show that a simple reduction of FBS concentration can achieve these goals, reduce costs and improve data consistency.

PA031

How to Improve Institutional Website Content about Animal Research

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Abstract
In collaboration with the European Commission, EARA regularly conducts a study (the third edition was published in 2024) to assess the openness and transparency of website content of those EU institutions that carry out biomedical research using animals. This presentation will discuss and explore the most important areas of good practice on website content and where improvement is needed.

An understanding of how to communicate to a general audience on animal research will help participants to see how websites - often the first impression anyone has of an institution - can be

used to deliver impactful messages on innovation, NAMs, 3Rs, severity and welfare, as well as focus on the most important arguments for the continued use of animals and why different species are used. Using clear, non-technical language can also help inform how we discuss ethical concerns with our colleagues and friends.

The presentation will put into context why there is a need for clear and effective website content on the use of animals in research and the progress of innovation to find suitable alternatives to animals; highlight the six ways that institutions can create robust and effective communications content, using examples from institutional websites, including content on culture of care, welfare and severity; use examples of good practice from websites and explain the practical steps that participants can take to improve website content at their own institution.

End

PA032

Education and Regulation in Animal Research: Ethics, Skills, and Responsible Practice

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Abstract
The module Knowledge in Laboratory Animal Science is part of the training program for Biotechnicians at our University. It provides biotechnology students with a comprehensive foundation in animal experimental research, focusing on both theoretical and practical skills. This module emphasizes the development of competencies for responsible and effective animal research, with a particular focus on preparing, executing, and defending applications to the Central Committee for Animal Experiments (CCD).

The CCD is the national body responsible for evaluating and approving animal research permits under the Dutch Experiments on Animals Act (Wod), which imposes strict conditions on the use of animals in scientific research. Students systematically learn to navigate ethical dilemmas, assess discomfort, and apply welfare guidelines according to Dutch Law.

The module integrates various facets of laboratory animal science, including genetics, housing conditions, welfare considerations, and statistical methods, all of which are covered through focused workshops. Students prepare for each session with literature and quizzes and consolidate their knowledge through assignments tied to their final project: drafting an official CCD application. Practical exposure includes field visits to research centers working with zebrafish, rodents, and primates, alongside engagement with critical perspectives, such as those from the Dutch Society for the Replacement of Animal Testing (Proefdiervrij). This ensures students develop a balanced understanding of the scientific, ethical, and regulatory aspects of animal research.

This module supports the shift toward refinement, reduction, and replacement (the 3Rs) in animal research and contributes to training professionals who are not only technically skilled but also ethically conscious.

PA033

"Animal Research: What, How, and Why". A Transmedia Approach to Shape Public Awareness

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Abstract
In Switzerland, public referendums aiming at banning animal research periodically threaten biomedical science and the pharmaceutical industry. In this context, experts in the field have noticed how the Swiss population remains largely unaware of the ethical and legal principles governing animal experimentation, making conscious voting uncertain.

Our project seeks to enhance transparency, curiosity, and awareness of animal research among the population using a transmedia approach.

First, we created a video podcast titled "Animal Research: What, How, and Why". In the initial videos, animal experimentation specialists illustrate the key concepts of ethical animal research in accessible terms. These include: what is animal research and its necessity, the 3Rs principles, and the relevant legislation. A team of researchers and experts in communication defined the video content and format, ensuring that the language and visual elements are suited to the general Swiss public. The videos are freely available across various social media and communication channels.

The first two videos collected over 15,000 views and 200 interactions (shares/likes) across different platforms. Additionally, they were presented at schools as well as national and international events targeting both researchers and the public. Moreover, a pilot study, involving a pre- and post-viewing questionnaire, demonstrated the effectiveness of this strategy in raising public awareness.

Our next steps include creating an interactive website and deploying mobile installations across public spaces to further disseminate our content. Furthermore, we plan to engage directly with the public during science-related events, collecting additional feedback and questions to be addressed in our future videos.

PA034

Decision Making Tools Enabling Methodological and Relevant Ethical Review of Study Protocols

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[2]*Charles River, Lyon, France*

Abstract
For laboratories using umbrella project licenses, the ethical assessment of all study protocols is important to ensure

compliance with regulations and 3Rs expectations. However, it represents significant workload and a challenge for ethical committee members to extract key information and make informed decisions. To assist with protocol review, a blood calculator sheet, vehicle tolerability database and dosing volume recommendations are already available. In addition, the following tools were created to help ethical commitee members achieve consistent and thorough reviews:

A checklist defining the items to be reviewed: study objectives, test item/vehicle, number of animals, housing, dose levels/volume, clinical follow-up/humane endpoint, blood sampling scheme and euthanasia method. For each item, a drop-down menu is proposed to decide whether the item complies with internal standards and/or with claimed project license, minimizes animal use and pain and is well justified.

A decision-making guidance defining criteria for giving disapproval, approval with recommendations or approval. For example, single housing without appropriate justification or selection of dose levels that would most likely result in excessive toxicity would trigger disapproval. Identification of refinements that could reduce severity or risk for animals such as staggered start or more specific humane endpoints would trigger approval with recommendations.

These tools allow a relevant and methodological ethical assessment and facilitate identification of critical aspects of the experimental design that could negatively impact 3Rs. It also increases decision consistency within the ethical committee, awareness of study directors on ethical expectations and enables better acceptance of the decision.

PA035

Revisiting the IQ CRO Assessment Tools to Align Assessment of Animal Welfare Due Diligence

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Abstract
The International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) is a not-for-profit organization of pharmaceutical and biotechnology companies (sponsors) with a mission of advancing scientifically driven standards and regulations for medicinal products worldwide. Contract Research Organizations (CRO) are critical partners providing in-vivo support for pre-clinical development. Both parties, the CROs and the sponsors, seek to support animal welfare and the 3Rs1. The CRO Outreach working group was created to foster collaboration between IQ member companies and the contract research organizations (CROs) with whom they partner. The working group collaborated on, piloted at their member companies, and released a risk assessment toolkit to assess animal care and use programs at partner CROs. The intent of the toolkit was to align sponsor animal care and welfare program assessments, decrease work burden on both parties, and focus resources on aligned advancements in animal care and welfare at the CROs. The Comprehensive Assessment Tool enables the review of housing, husbandry, veterinary care, ethical review, regulatory compliance, accreditation status and implementation of the principles of the 3Rs. An abbreviated version, the Low-Risk Assessment Tool, was also made available. Final versions released in 2019 were first publicized in 2020[2]; a time during which in person assessments were severely scaled back. It is time to reconsider the IQ CRO Assessment tools and consider the benefit of aligned animal welfare due diligence.

PB001

Magnetic Resonance Imaging for Blood-brain Barrier Dysfunction Early Detection: Improving Non-invasive Following-up

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Abstract
Magnetic resonance imaging (MRI) is widely used in neuroscience studies. This allows the evaluation of brain injuries at different time points in the same animals, following the reduction principle and enhancing the accuracy of the experiment. In this sense, blood-brain barrier (BBB) dysfunction plays a key point in devastating diseases such as stroke. Thus, the main objective of this study was to apply the gadolinium-enhanced MRI to detect BBB dysfunction at the initial stages in stroke-prone renovascular hypertensive rats (RHRSP).

Hypertension was induced in 12 male Sprague–Dawley rats using the method of two-kidney, two-clip. Systolic blood pressure was measured by the indirect tail-cuff method previously and weekly. An MRI T2 map was performed weekly to evaluate morphological brain alterations. A T1-weighted gadolinium-enhanced MRI was performed before and every two weeks after hypertensive induction. Gadolinium was injected via the tail vein using a syringe pump programmed to dispense it according to a 20-minute step-down infusion.

Systolic blood pressure was significantly higher in the RHRSP group since the first post-surgical week (134.1 ± 16.6 vs 113.5 ± 11.4 mmHg; $p = 0.041$). 3 of the 6 RHRSP rats showed cerebral ischemic lesions and only one of them showed cerebral hemorrhage. Finally, T1 maps showed an increase in BBB leakage measured by gadolinium extravasation into the whole brain of RHRSP rats at 4, 10, and 16 weeks after surgery.

In conclusion, this study shows that gadolinium-enhanced MRI can detect BBB leakage throughout the establishment of the hypertensive state in RHRSP rats.

PB002

Is the Microbial Status an Extrinsic, Intrinsic, or Intermediate Influence on Experimental Animals?

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Abstract
Living entities composed of the host and its associated microbial communities, such as laboratory animals, are defined as holobionts. The host genotype and its microbiome drive together as a metagenome the holobiont phenotype, with the microbiome itself as a well-recognized source of phenotypic variation. Multiple environmental (diet, light-dark cycles, etc.) as well as host-related factors (genotype, maternal effect, etc.) contribute to microbiome development, raising the question of whether the microbiome of experimental animals represents an extrinsic, intrinsic, or intermediate influence.

To study the impact of host genotype and maternal legacy on microbiota shaping, we transferred a mix of C57BL6/J and BALB/c embryos into B6CF1 recipient mice to obtain mice inoculated with an identical microbiome at birth. We then analysed the development of the offspring's colon and skin microbiomes over six generations in a constant environment. Our data show that, under controlled environmental conditions, "host genotype" is the main microbiome-driving factor along multiple generations. This is reflected in genotype-specific differences in the taxonomical composition and abundance of specific taxa, such as *Akkermansia muciniphila*, a known phenotype-influencing pathobiont. Moreover, the "maternal legacy" effect cannot be disregarded, especially in earlier generations.

Overall, our study supports the idea that the host does not leave its microbial status to chance, but controls it through host specific microbiome-shaping mechanisms. This advises that the microbiome of experimental animals is an intermediate factor that has both intrinsic and extrinsic components and praises an important refinement potential in the careful selection of the appropriate metagenome for each particular experiment.

PB003

Evaluation of a Prolonged Adaptation Period on Mouse Grimace Scale Baselines

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Abstract
For the Mouse Grimace Scale (MGS), a frequently used method to assess pain in laboratory mice, changes in facial expression are categorised into facial action units that relate to pain intensity. Therefore, healthy, pain-free mice should score with "zero" or very low baselines. However, elevated MGS baseline values are often reported, but the underlying reasons are unclear.

We hypothesised that the procedure for taking the footage for MGS may be a cause of discomfort for the animals due to the usually necessary transfer into observation boxes. Therefore, we wanted to analyse whether a prolonged adaptation period to the procedure may serve as a refinement.

For this, female C57BL/6J mice were adapted to the MGS procedure by separately being placed into the respective observation boxes for increasing durations over 3 days. Afterwards, the regular MGS procedure was performed at indicated time points. Two scorers analysed the facial action units orbital tightening, ear position, nose bulge and whisker change with the help of a semi-automated software. However, statistical analysis revealed that a prolonged adaptation period did not lead to reduced baseline scores over time.

In conclusion, our findings suggest that the applied adaptation strategy did not affect MGS baseline values. However, we did not assess further and more elaborated training and adaptation strategies in this set-up. Therefore, further studies are required to assess elevated MGS baseline scores and consequently increase the quality and stability of the achieved data as well as animal welfare.

PB004

Severity Assessment in the DSS-Colitis Model under Metamizole Pain Therapy

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Abstract
A commonly used mouse model of ulcerative colitis is the dextran sodium sulphate (DSS)-colitis model, which results in weight loss, softening of stool up to diarrhoea and abdominal pain. In terms of refinement, this study aimed at reducing the severity in this model through pain therapy with metamizole.

Ten-week-old, female C57BL/6J mice received DSS via drinking water over 5 days and controls received water only. The mice were then treated with metamizole (200 mg/kg/d) via the drinking water for an additional 5 days, while corresponding sub-cohorts were left untreated. For severity assessment, changes in bodyweight, posture, stool consistency, wheel running and Mouse Grimace Scale (MGS) were analysed.

Control mice, which were water or water/metamizole treated, showed no clinical signs of illness or changes in wheel running activity and MGS. In contrast, DSS/water and DSS/metamizole treated mice showed similar loss of body weight up to $11.2 \pm 6\%$ (DSS/water) and $12.8 \pm 7\%$ (DSS/metamizole) as well as signs of

illness like hunched posture and softened stool or diarrhoea. Wheel running performances also showed a similar decrease, in DSS/water treated mice up to 76 ± 20% and in DSS/metamizole up to 82 ± 17%. We found no statistically significant differences between untreated and metamizole treated animals suffering from DSS-colitis. Interestingly, none of the groups showed elevated MGS.

These data indicate that metamizole treatment via the drinking water did not reduce the severity in the DSS-colitis mouse model. Therefore, other refinement strategies should be addressed in future studies.

PB005

Refining Blood Sampling in Rodents - Reflections From a FELASA Working Group

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Abstract

Blood sampling is a common procedural step in rodent studies, and over the past few decades, advancements in techniques have made it more refined and sophisticated. With the introduction of Directive 2010/63/EU, which legally supports the 3Rs (Replacement, Reduction, and Refinement), there is growing awareness among researchers about the need to improve experimental techniques, especially in terms of animal welfare. Despite these developments, there is still considerable potential for further refinement. Recently, new blood sampling techniques have been proposed for which dissemination has been sub-optimal. Additionally, some studies have described new advantages and disadvantages of established techniques. While the variety of available methods and related data are positive developments, it can also complicate the decision-making process for selecting the most suitable technique.

On a different topic, it is important to recognize that effective blood sampling involves more than just selecting the best puncture site and technique. Factors such as sample volume, frequency of extraction, sample quality, and the impact of the procedure on blood parameters must also be carefully considered.

This FELASA Working Group is dedicated to making the refinement of blood sampling in rats and mice more accessible and practical. With its Report, the Group aims to provide tools and tips for selecting and utilising the most appropriate methods for each specific study goals while enhancing animal welfare and the quality of scientific outcomes.

PB006

Refined Tamoxifen Administration in Mice Via Encouraged Voluntary Consumption

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Abstract

Drug administration in preclinical rodent models is essential for research and the development of novel therapies. Existing compassionate administration methods are mostly incompatible with water-insoluble drugs such as tamoxifen or do not allow for precise timing or dosing of the drugs. For more than two decades, tamoxifen has been administered by oral gavage or injection to CreERT2–loxP gene-modified mouse models to spatiotemporally control gene expression, with the numbers of such inducible models steadily increasing in recent years. Animal-friendly procedures for accurately administering tamoxifen or other water-insoluble drugs would, therefore, have an important impact on animal welfare. To encourage voluntary drug intake by mice, we formulated palatable solutions compatible with water-insoluble drugs, such as tamoxifen, that can be administered using a micropipette. We evaluated the acceptance of the new formulations by mice during training and treatment and assessed the efficacy of tamoxifen-mediated induction of CreERT2–loxP-dependent reporter genes. Sweetened milk but not syrup-based formulations were statistically non-inferior to oral gavage or intraperitoneal injections in inducing CreERT2-mediated gene expression. Serum concentrations of tamoxifen metabolites confirmed the lower efficacy of syrup- as compared to sweetened milk-based formulations. We found dosing of the sweetened formulations with a micropipette to be more accurate than conventional oral gavage or injection methods, with the added advantage that pipette feeding requires little training for the experimenter. The new palatable solutions encourage voluntary consumption of tamoxifen without loss of efficacy compared to oral gavage or injections and thus represent a refined administration method.

PB007

Evidence-based Severity Assessment and Validation of Refinement Measures in Rodent Epilepsy Models

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Abstract
For ethical and legal reasons and, to implement the 3R principles in animals-based research, evidence-based welfare and severity assessment is essential. Within our research group, suitable parameters for an objective severity assessment are identified and validated. Subsequently, composite measure schemes are used as a basis for evidence-based comparative severity assessment. In our studies, we focused on rodent epilepsy models.

Various behavioral and biochemical parameters were assessed in rodent kindling and post-status epilepticus models. In some sub-studies, locomotor activity, heart rate, and heart rate variability were analysed using telemetric recordings. Selected data sets were then applied to a bioinformatic workflow including correlation and principal component analysis and *k*-means-based clustering.

Parameters that proved to be sensitive indicators of animal distress included burrowing behavior, saccharin preference, fecal corticosterone metabolites, heart rate, and heart rate variability. Additionally, grimace scores and nestbuilding behavior were affected in the early post-surgical phase in both species. The following cluster analyses enabled the allocation of individual animals to different severity levels.

In conclusion, we identified several valuable and sensitive parameters suitable for application in epilepsy models. Interestingly, cross-correlation analysis indicated that telemetric parameters do not provide additional information. Instead, a combination of behavioral and biochemical is sufficient to capture sensitive information about an animal's distress and the affective state without previous invasive procedures. With regard to the bioinformatic approach, we confirmed the suitability of the composite measure schemes for evidence-based severity assessment across different models.

Supported by Deutsche Forschungsgemeinschaft (Severity Assessment in Animal Based Research, FOR2591, PO681/9-1,-2 and-3).

PB008

Establishment of an Environmental Health Monitoring Strategy Detecting Theiler's Murine Encephalomyelitis Virus (TMEV)

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Abstract
Routine health monitoring (HM) is the fundamental basis for preserving animal health and ensuring research validity. Theiler's Murine Encephalomyelitis Virus (TMEV) is a single-stranded RNA cardiovirus, either causing asymptomatic enteric infections or neurological disease. Routine detection is typically performed using indirect serological methods. With increasing utilization of molecular techniques such as quantitative-Polymerase-Chain-Reaction (qPCR) analyses, it became common to establish environmental HM (EHM) strategies to reduce the number of animals used while increasing diagnostic success, particularly in individually ventilated cage (IVC) systems. However, validated EHM protocols for TMEV remain unavailable to the public. This study aims to validate an EHM strategy for TMEV detection in mouse colonies.

To this purpose, ten C57BL/6JRj mice were oronasally infected with the GDVII TMEV strain and kept in pairs of two with a contact-sentinel (RjHan:NMRI). Additionally, one corresponding cage with soiled-bedding sentinels (RjHan:NMRI) were used to monitor each IVC rack. Four weeks p.i. environmental- as well as animal-derived samples were analyzed by qPCR and compared to traditional serology.

While serology detected TMEV-infection in 80% of the inoculated mice, no antibodies were detected in the sentinels. EHM was superior compared to the animal-derived sampling strategy, which showed diagnostic success exclusively in mice showing clinical symptoms. The Sentinel-Free-Soiled-Bedding (SFBS) filters outperformed all other sample types with 100% detection rate. In contrast, analysis of the exhaust air dust (EAD) filters only showed 25% detection rate.

Thus, an EHM approach using SFBS-filters supplemented by a systematic qPCR follow-up of clinically sick mice was proven suitable for routine HM.

PB009

Validation of a Metagenome-Based Environmental Health Monitoring Strategy to Increase Animal Research Validity

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Abstract
Health monitoring (HM) of laboratory rodents is essential for quality assurance in animal facilities. Besides pathogens, opportunists and individual study confounders, there is profound evidence, that also commensals and the microbiota composition influences research data. Consequently, microbiome characterization should be included in HM strategies to increase the scientific validity of projects. However, these analyses are, until now, not part of routine programs. Therefore, the aim of this project is to establish an innovative HM methodology, which involves the metagenome analyses of the exhaust air dust (EAD) of individually-ventilated-cage systems.

To this purpose, 23 EAD-filters were analyzed by quantitative Real-Time-Polymerase-Chain-Reaction (qPCR) and Next-Generation-Sequencing (NGS) and the diagnostic sensitivity and specificity were calculated. Filters spiked with defined bacterial suspensions and EAD field samples from existing colonies were used as positive and negative controls. Furthermore, a full microbiome profiling was performed comparing EAD-filters and feces as sample matrices.

Test accuracy of NGS was comparable to the qPCR used as a gold standard. While most agents were successfully detected based on the relative number of read counts, precise diagnostics of *Staphylococcus aureus* and *Klebsiella oxytoca* required using specific genome coverage ratios as thresholds to increase the diagnostic specificity.

As expected, microbiome analyses of EAD-filters and feces show distinct alpha-diversity of bacterial communities, whereas both sample matrices were suitable to detect differences in beta-diversity between separate units.

Altogether, the establishment of this innovative HM approach contributes to the 6R-concept, which addresses besides animal welfare also the scientific validity of research.

PB010

Comparison of Double Chamber and Whole-body Plethysmography in Guinea Pig Cough Assessment

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Abstract
Objective: This study aims to compare the efficacy of double chamber plethysmography (DCP) and whole-body plethysmography (WBP) in evaluating citric acid-induced cough responses in guinea pigs.
Methods: Sixteen specific pathogen-free (SPF) and sixteen conventionally-bred (CON) guinea pigs were exposed to 0.4M citric acid aerosol. Both DCP and WBP methods were used to measure cough frequency and latency to the first cough. Statistical analyses were performed to identify significant differences.
Results: WBP yielded significantly higher cough counts and shorter latencies compared to DCP for both SPF and CON animals. For SPF guinea pigs, WBP cough counts were 13±9 vs. 2±3 for DCP, with latencies of 59±6 s vs. 159±14 s ($p < 0.0001$). For CON animals, cough counts were 14±8 vs. 5±5, and latencies were 77±4 s vs. 112±12 s ($p < 0.0001$). The unrestrained nature of WBP allowed more physiologically relevant aerosol distribution, influencing cough responsiveness.
Conclusion: The study demonstrates that methodological differences between DCP and WBP substantially affect cough outcomes. WBP, which enables unrestrained measurements, is more reliable and clinically relevant for assessing the cough reflex. These findings underscore the importance of standardizing cough research protocols to enhance reproducibility and translational relevance. Future studies should account for variables such as microbiota status and experimental setup to minimize variability in preclinical respiratory research.
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PB011

Ultrasound Imaging to Reduce Severity in Animal Research

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Abstract
Since its beginnings, ultrasound technology has provided insight in both human and veterinary medicine on the physiological and pathological state of subjects. It is now largely available and adapted for small animals such as rodents. Ultrasound imaging is a powerful tool to assess in vivo and in a painless method the effect of treatment and/or genetic modifications in laboratory animals. In this presentation, we will show how this technology can help researchers and veterinarians reduce severity in animal research. First, we will present the case of the left ventricular systolic function evaluation after induced myocardial infarction in rodents and how echocardiography can prevent the unnecessary suffering of animals too impacted by the surgery. We will also show an in vivo method to evaluate hepato-pulmonary syndrome in genetically modified mice using contrast enhanced ultrasound. Finally, we will explain how ultrasound helped in the early detection of liver metastasis in a rodent model of colon adenocarcinoma.

PB012

Wild Animals in Experimental BSL-3 Studies: New Challenges Working with American Mink (*Neogale vison*)

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Abstract
Europe has witnessed a surprising increase in the number of highly pathogenic avian influenza A(H5N1) virus cases in various bird species and mammals. Specifically, in October 2022, an HPAI A(H5N1) virus infected farmed minks in Galicia, Spain, and subsequent investigations indicated apparent mink-to-mink transmission of the virus within the affected farm. This outbreak marks a turning point in the epidemiology of avian influenza viruses and their zoonotic potential.

The aim of the ISIDORE project (TNA) was to evaluate the transmission route and pathogenicity of a highly pathogenic avian influenza H5N1 strain adapted in American mink (*Neogale vison*) mammals, addressing the challenges of using wild-caught animals for research while prioritizing ethical considerations.

Thirty-six American minks were housed in our BSL-3 facility for 25 days, presenting unique challenges in animal welfare and biosafety protocols. We implemented environmental enrichment strategies, including drapes, nesting materials, toys, and nutritional supplements. Daily clinical observations focused on respiratory symptoms, with body weight, temperature, blood or oropharyngeal samples collected in different study days under sedation.

Our study successfully adapted housing and handling protocols for this non-traditional research species. The minks acclimatized well to the environment and manipulation activities, demonstrating the potential of American mink as a model for infectious disease studies. This work contributes to the refinement of research methods using wild animals and advances our understanding of H5N1 transmission in mammals. The successful adaptation of American mink for H5N1 research provides valuable insights for future studies on zoonotic diseases, balancing scientific needs with animal welfare considerations.

PB013

Reducing Experimental Severity: The Role of Oxyletpro - Physiocage System in Animal Research

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Abstract
Technological advancements in animal research are improving scientific outcomes and animal welfare by minimizing stress and intervention, in line with the 3Rs principles, as external stressors can significantly impact physiological and behavioral results. The OxyletPro - Physiocage system represents a significant advancement over traditional systems. It provides a wide range of parameters (respiratory metabolism, intake, activity/rearing), high-quality data, reproducibility, and high-throughput phenotyping with parallel cage monitoring. A key advantage of this approach is the ability to monitor animals under controlled conditions within their home cages, thereby eliminating human bias and standardizing the environment. This is particularly important because activities such as cage changes and handling can disrupt behavior, circadian rhythms, and sleep patterns, while also inducing stress. In mental health research, for example, digital metabolic cages reduce the need for repeated handling, which effectively lowers stress and minimizes the severity of experimental procedures. This refined methodology not only enhances animal welfare by reducing distress, but it also ensures the most precise and consistent data collection. By minimizing physical interventions, the OxyletPro system significantly reduces the severity of procedures involving laboratory animals, contributing to both the refinement of experimental practices and the advancement of ethical standards in animal research.

PB014

Biosecurity and ART in Mouse: Literature Review and Ten Years Retrospective Activity Analysis

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Abstract
The advancement of assisted reproduction technologies (ART) has revolutionized the manipulation of mouse pre-implantation embryos, opening new avenues for genetic research and powerful 3Rs approaches for managing genetically engineered animal colonies. However, these technologies also introduce biosecurity risks that must be carefully managed. A primary concern is the potential for contamination during the production and storage of embryos, with possible contaminants including viral, bacterial, or parasitic agents. Contaminants may be introduced during in vitro fertilization (IVF) or through gametes and embryos themselves. This risk could be particularly significant with microinjection or IVF-derived technologies such as laser-assisted IVF, which create intentional breaches in the zona pellucida of pre-implantation embryos, thereby reducing their natural protection barrier.

This presentation will review biosecurity risks linked to the use of pre-implantation embryos and provide a retrospective analysis of ART outcomes over the past 10 years on our European reproductive and genetic engineering platform. Our experience demonstrates that these technologies do not compromise the health status of generated mouse lines. Furthermore, they offer numerous benefits for managing colonies of genetically modified and mutant mice.

PB015

Controlling Genetic Integrity in Mouse Research Models: The GenoCheck Service

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Abstract
When reusing published genetically modified mouse models, researchers encounter complexities due to insufficient access to critical genetic information. Details on genetic background and mutations are often lacking, complicating genotyping and impacting the interpretation of scientific results.

For instance, studies have shown that the verified genetic background frequently does not match the indicated one (1) Similarly, many models exhibit actual mutation functions that differ from the expected ones (2). Inadequate control over these factors compromises research quality by hindering reproducibility and leading to unnecessary experiments and the use of animals, which could be avoided with adequate prior scientific information (aligning with the 3Rs principle).

In line with the ARRIVE guidelines for documenting laboratory animals, the Laboratory Animal Genetic-Reporting (LAG-R) framework was developed to standardize and improve genetic information (3). These recommendations have been co-signed by numerous experts, societies, and infrastructures. Their adoption should facilitate the use of genetically modified animals. However, critical genetic information is often missing when importing or using mouse models. To address this, we developed GenoCheck, a new service providing initial verification of this essential information.

Our presentation will highlight common genetic deviations in genetically modified mouse models and demonstrate how GenoCheck can mitigate risks in scientific projects.

PB016

Breaking the Year Barrier Ethically: Accelerated Backcrossing in Mice Using IVF and SNP Techniques

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Abstract
We introduce a novel method for accelerated backcrossing in mice, integrating in vitro fertilization (IVF) and single nucleotide polymorphism (SNP) analysis. This approach significantly shortens the backcrossing process to less than a year, resulting in a 40% reduction in the number of animals used and a decrease in cage occupancy.

IVF facilitates the rapid production of progeny, while SNP analysis allows for precise genetic monitoring and selection. By combining these techniques, we streamline the traditional backcrossing timeline without compromising genetic fidelity.

Backcrossing is an essential process in genetic research, used to transfer a specific mutation or genetic trait from one genetic background to another. It allows researchers to study the effects of specific genes in different genetic contexts, enhancing the reproducibility and robustness of experimental outcomes. This method ensures that any observed phenotypic changes are due to the introduced mutation, rather than other genetic variables.

The traditional approach, even when using SNP analysis to accelerate the process, remains too slow and requires a large number of animals. This results in extended timelines and higher resource utilization, impacting both efficiency and ethical considerations.

Our results demonstrate the method's efficiency in conserving animal resources, aligning with the 3Rs principle (Replacement, Reduction, Refinement). This method has substantial implications for genetic research, potentially setting a new standard for mouse model development in laboratory settings.

PB017

Enhancing Mouse Research Model Colony Management and the 3Rs through Sperm and Blastocyst Genotyping

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Abstract
Genetically modified mice are vital in fundamental research and as models for human diseases. Traditionally, confirming their genotype involves generating live pups, requiring significant animal use, time, cage space, and resources. However, sperm and preimplantation embryo genotyping offer a promising and ethical alternative.

Our collaborative efforts between the genotyping and reproductive and genetic engineering platforms have developed robust automated PCR protocols for genetic analysis using fresh or frozen-thawed sperm and individual preimplantation embryos. A single straw of sperm provides sufficient DNA for PCR, producing fragments up to 1.5 kb. These samples can be stored for weeks without losing quality. Similarly, the crude extract from a single blastocyst suffices for genotyping, with amplification products up to 320 bp, and can be stored or shipped for days.

This collaboration significantly enhances cryostock validation, saving numerous animals per strain and reducing the need for cage space. The method is also beneficial for evaluating and optimizing gene editing technologies like CRISPR/Cas9, particularly for complex alleles with low efficiency rates. Moreover, sperm genotyping enhances ES-based transgenesis by early identification of chimeric males capable of germline transmission (GLT). Using digital droplet PCR and automated analysis, we predict germ-line transmission, allowing IVF with only one male showing the best parameters, saving around 50 animals per model, and shortening project timelines by 3 to 6 months.

Our integrated approach not only aligns with ethical research principles by minimizing animal use but also streamlines processes and optimizes resource utilization, showcasing the strength of our collaborative platform.

PB018

Managing Genetically Engineered Animals Through the Lens of the 3Rs

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Abstract
The preclinical scientific community has long supported the principles of the 3Rs (Replacement, Reduction, and Refinement). For many years, Charles River has embraced these principles, taking responsibility for all aspects of our work and driving progress for both patients and animals that depend on our research. This presentation focuses on the management of genetically engineered rodent models through the lens of the 3Rs.

Key topics include the development and implementation of efficient embryology and genotyping refinements, highlighting how these techniques can reduce the number of animals used and improve the quality of research outcomes. Additionally, pioneering techniques for model generation are explored, showcasing how cutting-edge methods can replace more invasive procedures and refine the use of genetically engineered models. Non-invasive sampling methods for genotyping, based on tears, feces, hair sampling, and oral swabbing, will also be presented. Finally, powerful digitalization solutions for animal identification and colony management will be discussed.

By integrating these principles into the management of rodent models, Charles River demonstrates a commitment to ethical research practices. This approach not only enhances animal welfare but also improves the reliability and reproducibility of scientific data, ultimately benefiting the broader scientific community and advancing biomedical research. This presentation aims to share these advancements and inspire continued adherence to the 3Rs within the research community.

PB019

Scoring Animal Welfare to Determine Cumulative Maximums

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Abstract
The laboratory animal community is actively seeking practical methods to inform cumulative welfare assessment and endpoint determination. Indeed, in Canada, guidelines require animal care committees to set policies describing cumulative endpoints. The problem. . .we currently do not have any practical methods or strategies to assess cumulative welfare impact or to determine how and when we have reached cumulative maximums.

Together with Drs Sally Thompson-Iritani (Asst. Vice Provost, Animal Care, Outreach and 3R's University of Washington and Sarah Wolfensohn (Emerita Professor, School of Veterinary Medicine, University of Surrey, and creator of the Animal Welfare Assessment Grid (AWAG) system and software), we have been investigating how to use welfare assessment software systems to calculate cumulative welfare assessment scores as well as to practically implement these scoring systems to determine cumulative welfare maximums.

In this session we will discuss what cumulative maximum welfare scores are, why they are so important and how we can go about scoring these practically. The overall goal will be to describe the use of the cumulative welfare scores to create practical scoring systems that can be used by Animal Care Committee to create scoring policies and systems for your institutions to demonstrate the implementation of refinements and welfare management.

PB020

Feedback on the Use of Monoclonal Antibodies in Dog and Cat Pain Management

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Abstract
Veterinary pharmaceutical specialties based on monclonal antibodies currently only have two applications in domestic carnivores: the management of pain related to osteoarthritis in dogs (Librela®) and cats (Solensi®), as well as the management of pruritus related to canine atopic dermatitis (Cytopoint®). Clinical trials have been conducted, but we currently have little feedback on the results obtained in the field in terms of efficacy, but also on the methods of use by veterinarians. Thus, this work consisted, on the one hand, of collecting veterinarians' practices with regard to drugs based on monoclonal antibodies, and on the other hand of collecting the results obtained on their patients. The practices of using monoclonal antibodies mainly have common points and some differences: Librela® and Solensia® are frequently used as first-line treatment in the management of pain related to osteoarthritis, especially in animals with organ failure. They are often used in combination with other therapies. Concerning their action, they are considered very effective, with maximum effectiveness most often achieved after two injections for Librela®. However, the response to these treatments presents interindividual variability and the effectiveness seems to decrease during the injections. All the elements are detailed in the veterinary thesis defended by Mr. Théo Doffe, in November 2023.

PB021

Oral Acepromazine Pre-treatment in Rabbit Kits Prior to Euthanasia via Intraperitoneal Pentobarbital Injection

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Abstract
The issue of euthanasia represents a pivotal aspect of animal care, and it is important to recognise that this process can be distressing for both the animals themselves and the technicians involved. The sensitivity of rabbit kits necessitates particular consideration when considering the method of euthanasia. An intraperitoneal pentobarbital injection has been identified as a potential source of significant stress for these young animals. Moreover, if prior sedation is necessary in the Summary of Product Characteristics of pentobarbital products intended for animal euthanasia, many sedatives require subcutaneous or intramuscular injections, which can result in additional stress and/or pain prior to the euthanasia process itself. The results of our study demonstrate that pre-treatment with the tranquilizer acepromazine via the oral route can improve animal welfare by reducing anxiety and distress during euthanasia. This non-invasive pre-treatment has been demonstrated to smooth euthanasia process, while also conferring benefits to the mental well-being of the personnel involved in the procedure. Furthermore, the incorporation of non-invasive acepromazine administration into euthanasia protocols may serve to enhance the Culture of Care within institutions, thereby demonstrating a commitment to ethical standards and humane treatment. In conclusion, the use of oral acepromazine as a pre-treatment for rabbit kits prior to euthanasia via intraperitoneal pentobarbital injection represents a notable advancement in veterinary care. This method enhances animal welfare and supports caregivers' emotional health, thereby facilitating a more compassionate end-of-life care approach.

PB022

Comparison of Different Surgical Techniques of Vasectomy in Mice Associated with Pain Control Protocols

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Abstract
With the aim of evaluating different pain control protocols, associated with longitudinal and transverse incision techniques for abdominal access, 48 male B6D2F1 mice, aged 8 weeks and older, were used. They were divided into groups according to surgical technique and pain control protocol ($n = 8$/group). Group LCT (Longitudinal+ketoprofen+tramadol), LC (Longitudinal+ketoprofen), LT (Longitudinal+tramadol), TCT (Transversal+ketoprofen+tramadol), TC (Transversal+ketoprofen) and TT (Transversal+tramadol). The LCT did not present a significant difference ($p = 0.61$) between the mean weight of the animals. The LC showed a difference between days 1 and 3 ($p < 0.05$). LT was different between days 1 and 2 ($p < 0.05$) and 1 and 4 ($p < 0.05$). The TCT showed difference between days 1 and 3 ($p < 0.01$), 1 and 4 ($p < 0.05$), 2 and 3 ($p < 0.01$), 2 and 4 ($p < 0.01$) and 2 and 5 ($p < 0.05$). The TC was different between days 1 and 3 ($p < 0.01$), 1 and 4 ($p < 0.05$), 2 and 3 ($p < 0.01$), 2 and 4 ($p < 0.01$) and 2 and 5 ($p < 0.05$). TT, there was a significant difference ($p < 0.05$) only between days 1 and 2. When comparing the weight variation between the surgical techniques, it was observed that between the LCT x TCT, there were greater mean weight loss in the TCT ($p < 0.05$). Between the LC x TC and LT x TT, there was no statistical difference ($p = 0.80$; $p = 0.6$). The groups of Transversal x Longitudinal techniques, there was a difference ($p < 0.01$) with higher means of weight variation in the transverse incision technique. It is concluded that the longitudinal surgical approach is less traumatic than the transverse one.

PB023

Transgenic and Archiving Module: Implementation and Enforcement of the 3Rs Principle

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Abstract
Transgenic and Archiving Module (TAM) is a key part of the Czech Centre for Phenogenomics (CCP). TAM provides complete service, from the initial gene-targeting design, generation of the rodent model, to the genotyping, breeding and archiving.

Implementation and enforcement of the 3R concept for each step of the whole process, from planning the experiment, selection of technology, harm-benefit analysis, husbandry conditions, colony management of genetically modified lines to actual procedures, are crucial for all three modules of CCP, except TAM also Animal Facility Module (AFM) and Phenotyping Module (PM).

TAM is responsible for the generation of novel genetically modified mice and rats using state-of-the-art technologies that allow the reduction of animal consumption in line with 3R principles. For example, CRE/FLP mediated allele conversions using AAV vectors enables the conversion from tm1a to tm1d in a single animal during IVF-based reanimation/rederivation from sperm, resulting in 80% of fully converted animals. Furthermore, mouse/rat model generation using programmable nucleases (TALEN, CRISPR/Cas9) using electroporation instead of microinjection reduces the number of donors animals, more than 90% of murine embryos continue their development.

In conclusion, before starting a new project a harm - benefit analysis is performed and also knowledge of veterinarians of AFM and scientists from TAM and PM of CCP is incorporated. The health and well-being of newly generated genetically modified lines are effectively monitored for clinical abnormalities, the number of animals used are continuously analyzed and measures that can enhance animal welfare are implemented.

PB024

Welfare Implications Related to Sex in a Rat Thoracic Spinal Cord Injury Model

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Abstract
Due to anatomical advantages in urination after injury, female rats are used in most spinal cord injury (SCI) studies. Nonetheless, it is becoming more and more advised to also include male rats to guarantee that the results apply to both sexes. Using dental burr-assisted laminectomy and conventional laminectomy techniques on Wistar rats, this study examined sex-related differences in SCI outcomes. SCI contusion was performed at the T11-T12 level in thirty-two rats (16 males and 16 females). Over a 28-day period, the study assessed how these techniques affected welfare, functionality, and histopathology. Study parameters included postoperative body weights and general clinical signs, open cage and home cage activity, fecal corticosterone levels, rat grimace scale (RGS), Basso Beattie Bresnahan (BBB) locomotor scores, and histopathology. The study found that male rats—despite refinement efforts—had significant welfare problems due to urine retention, including high levels of stress, increased pain, and adverse clinical symptoms. Male rats are essentially unsuitable for this SCI model because these issues persisted despite rigorous treatment and advancements in surgery. The welfare consequences for male rats were substantial despite the fact that histopathological analysis did reveal differences in the sexes injury patterns and healing processes. Even though the study obtained some crucial information regarding the progression of the disease, it concludes that male rats should not be used in this SCI model in order to protect animal welfare. It also highlights the need for sex-specific considerations in preclinical SCI research, further reinforcing the importance of animal welfare in scientific designs

PB025

Establishing a Mouse Model for Early-Stage MASLD: Refinement Through Diet Design

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Abstract
The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) is increasing worldwide, driven by diets high in fructose and fat. Early stages of MASLD are of particular concern as they are asymptomatic, often undiagnosed but reversible, making them critical targets for early intervention. Up to 25% of the world's population are affected by MASLD. Alarmingly, the disease is spreading to younger age groups, increasing time available for serious complications to develop. Despite this, preclinical models to test potential compounds that recapitulate these early changes while minimising experimental severity remain underdeveloped.

Preliminary data show that C57BL/6J and ApoE-/- mice fed a high-fat/high-fructose diet exhibit histological liver changes, lipid accumulation and signs of early inflammation, including increased expression of IL-1β and COX2. Mice also have elevated fasting serum glucose and cholesterol levels. Importantly, no significant fibrosis was observed, supporting the relevance of the model to early, reversible stages of MASLD. These findings suggest that the model effectively captures the hallmark features of early MASLD. The presence of lipid accumulation and early inflammation highlights its utility for testing therapeutic interventions at this stage.

This refined approach emphasises the translational relevance of the model to humans, while adhering to the 3Rs principles by prioritising mild disease phenotypes and minimising experimental severity. By avoiding the stress and adverse effects associated with advanced disease stages or highly harmful diets, such as methionine-choline-deficient diets, this model offers an ethical alternative. Further analysis will demonstrate its potential for investigating preventive measures and evaluating early therapeutic interventions.

PB026

Refinement of chimerism evaluation of humanized mice through digital PCR low blood volume analysis

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Abstract
Humanized immune system (HIS) mice contain human and murine immune cells. Analysis of chimerism in HIS mice is typically accomplished via flow cytometry of peripheral blood, but this requires collecting a 75 μL blood sample and access to a flow cytometer. Repeated bleeding can negatively affect the health of HIS mice, limiting serial analysis. In this study we hypothesized that detection of humanization could be performed accurately via small blood volume sample analyzed via digital PCR. Juvenile NOG-EXL female mice (n = 24) were myeloablated and engrafted with cord blood-derived human hematopoietic stem cells. At 10 weeks post-engraftment, peripheral blood was analyzed via flow cytometry after being stained with anti-murine CD45 and anti-human CD45 antibodies, and results compared to digital PCR analysis of human genes SRSF4, SF3A1, IPO8 and mouse B2m targets on extracted total nucleic acids from the same sample. Linear regression and mean of difference was calculated with GraphPad Prism. Tight correlation was found when linear

regression was analyzed, R2 = 0.9529. Mean of difference was 2.65% with flow cytometry reporting a higher percentage of human component. Despite this, the correlation coefficient (r) was 0.9762, p value (one tailed) <0.0001 indicating significant agreement between the two methods. Digital PCR offers a novel method for chimerism analysis, which uses much smaller blood volumes (as little as 10 μL) and can be performed on clotted or frozen samples, sample types unsuitable for flow cytometry.

PB027

From Caterpillar to Cat: Indirect Calorimetry as a Translational Method for Determining Metabolic Processes

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Abstract
Indirect calorimetry is a non-invasive method of measuring energy expenditure and substrate oxidation by analyzing oxygen consumption and carbon dioxide production. It has become an essential tool in both human and animal research, particularly in the study of energy metabolism, metabolic diseases, and the effects of interventions such as diets or pharmacological treatments. Its translational importance lies in the ability to generate comparable data between species and investigate physiological mechanisms in the context of preclinical and clinical research. This technique advances the understanding of the pathophysiology of metabolic diseases and supports the development of new therapeutic approaches. The data presented here, ranging from caterpillars to cats, provide a methodological insight into the technique of indirect calorimetry. We have described the metabolic processes in tobacco hornworm caterpillars (*Manduca sexta*) from the wandering phase to pupation and emergence. In mice, the pharmacological effects of a UCP3 uncoupler are demonstrated, while in cats, data on basal fasting metabolism and the postprandial phase are shown. This serves to illustrate just a small excerpt of the diverse applications of indirect calorimetry.

PB028

Focus on Severe Suffering - Picking the 'High-hanging Fruit'

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Abstract
There has been increasing commitment to reducing and avoiding 'severe' suffering, with some success. For example, there has been a 61% reduction in procedures causing severe suffering in the UK since 2014.

A number of factors have contributed to this. Many relate to experimental design, including earlier scientific and humane endpoints, better screening of animals and strain selection, use of models at earlier disease stages, better husbandry and support, and use of technology such as temperature transponders to identify clinical signs. Other, 'cultural' factors have also made a positive difference, e.g. better communication within teams, better training for animal technologists, and more involvement of Animal Welfare Bodies (AWBs), Animal Ethics Committees (AECs) and equivalent bodies.

However, some procedures currently appear harder to refine. According to national statistics on animal use, these 'high-hanging fruit' include animal disease models, batch potency tests and disease diagnosis. These account for a significant proportion of severe suffering, as do acute toxicity studies in the area of ecotoxicity. From a species perspective, the mouse can be most likely to experience severe suffering. For example, in the EU and Norway in 2020, 12.5% of all mouse use was severe, whereas 10% of all procedures were severe.

This presentation will explore potential approaches to avoiding and reducing severe suffering in procedures where this is currently difficult, identify ways of overcoming barriers to refinement, and discuss ethical issues around severity levels according to species and purpose.

PB029

Exploring NaV1.7 Blockade in Cough Suppression in a Guinea Pig Model of Allergic Rhinitis

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Abstract
Introduction: The most frequent cause of chronic cough, a severe clinical issue, is allergic rhinitis (AR). The cough hypersensitivity syndrome is linked to inflammatory agents' dysregulation of nodose Aδ-fibres and jugular C-fibres. There is a constant need to discover new, more effective antitussives because commercially available antitussives are frequently ineffective with many adverse effects. Recent developments in our knowledge of voltage-gated sodium channels (NaVs) have shown that subtype NaV1.7 may be an attractive target since it is implicated in the initiation and conduction of cough. This study aims to assess the effectiveness of the NaV1.7 sodium channel blocker PF-05089771 on cough in a guinea pig model of AR.
Methods: Guinea pigs were sensitized with ovalbumin (OVA) and subjected to repeated nasal OVA challenges (NCHs) to develop chronic AR. One hour after the 1st, 3rd, and 6th NCHs, cough was induced by inhalation of 0.4M citric acid aerosol. The tested OVA group was pre-treated with an inhaled NaV1.7 blocker (PF-05089771, 100 μM, 10min) before tussigen inhalation.
Results: Compared to the control group, chronic AR significantly enhanced cough reaction to 0.4M citric acid (11.5$\pm$2.11 vs.

5.9±1.20, $P < 0.05$, $n = 9$ for each group). The cough reflex was significantly inhibited by ≈ 75% after pretreatment with the NaV1.7 blocker (2.7±0.75 vs. 11.5±2.11, $P < 0.0001$) without affecting the respiratory rate.

Conclusion: Inhalation of NaV1.7 blocker resulted in cough inhibition in awake guinea pigs with AR.

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PB030

Investigating the Role of Hydrogen Sulfide in Osteomyelitis Using CSE Mouse Models

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Abstract

Osteomyelitis is a bone inflammation caused by pathogens like *Staphylococcus aureus* with anti-infective therapy being the primary treatment. However, increasing bacterial resistance highlights the need for novel therapies. Recently, cystathionine-γ lyase (CSE), an enzyme involved in hydrogen sulfide (H_2S) production, has emerged as a key immune modulator with anti-inflammatory effects. This study investigates the role of H2S in regulating infection and inflammation in primary and secondary osteomyelitis using *Cse*-deficient mice. Therefore, 72 ($n = 18$/group) 4-month-old, including both sexes, transgenic mice $Cse^{+/+}$ (wild-type with physiological expression) and $Cse^{-/-}$ (knockout mice) with either intramedullary inoculated of 10^3 Staphylococcus aureus cfu/mouse into the femur or injected of 10^7 *Staphylococcus aureus* cfu/mouse into the tail vein to induce bacteremia (secondary osteomyelitis). Survival was monitored by observing overall welfare and weight measurements for 21 days. Furthermore, bacterial loads were quantified in tissue samples isolated from sacrificed animals. Higher mortality and bacterial loads were observed in the bacteremia groups. Mortality in $Cse^{+/+}$ mice at 21 days was 5% in primary and 44.5% in secondary osteomyelitis (p-value = 0.02). In deficient mice, 5% fatality was noted in primary and 80% in secondary osteomyelitis (p-value < 0.0001). Statistically significant differences in bacterial loads were observed in the liver and kidney. In $Cse^{+/+}$ mice, the p-values were 0.008 for the liver and 0.003 for the kidney between primary and secondary osteomyelitis. Similarly, significant differences were observed in $Cse^{-/-}$ mice, with p-values <0.0001 for both tissues. In conclusion, H_2S seems to reduce systemic infection severity, warranting further research.

PB031

Analgesic refinement of a murine orthotopic xenograft model for acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is associated with a very poor prognosis in adulthood, with a 5-year survival probability of less than 25%. The use of clinically relevant animal models remains crucial. Continuous refinement of animal experiments, as part of the 3R principles, is still mandatory. Thequantification of efficacious analgesics represents an important aspect of animal model-specific refinement.

The orthotopic xenograft model was induced by intravenous injection of the human precursor ALL cell line SEM into immunodeficient NSG mice. When signs of pain and discomfort, as defined by the score sheet, were observed, either metamizole (3 mg/ml) or tramadol (1 mg/ml) was administered in the drinking water for analgesic treatment. Monitoring of animal welfare was performed weekly during leukemia progression and daily after initiating continuous analgesic administration, using body weight, burrowing behavior, nesting activity, clinical score, perianal temperature, liquid intake, and mouse grimace scale.

Following the start of analgesic treatment, a significant reduction in body weight and liquid intake was observed for both analgesics. A significant increase in the clinical score, as well as reduced burrowing and nesting activity, were noticed after starting metamizole administration but not for tramadol-treated mice.

Combining the data with the Relative Severity Assessment (RELSA) Algorithm revealed that oral metamizole treatment leads to a stronger impairment of health in mice compared to tramadol application. The opioid tramadol should replace metamizole as the analgesic compound of choice for the ALL-xenograft model to improve animal welfare in future studies.

PB032

Comparison of Analgesics in the Murine Bile Duct Ligation Model

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Abstract

The bile duct ligation (BDL) model is an established animal model for studying cholestatic liver diseases. However, it is associated

with pain and distress for the animals, highlighting the need for optimized analgesic treatment. This study aimed to evaluate the analgesic effect of metamizole, tramadol, and carprofen in the BDL model.

C57BL/6J mice were assigned to equally sized groups (N = 10) for treatment with either metamizole (3 g/L), tramadol (1 g/L), or carprofen (150 mg/L), administered daily via drinking water. A telemetric device (ETA-F10, DSI, St. Paul, MN) was implanted 22 days before bile duct ligation to monitor ECG, temperature, and locomotion. To evaluate distress and pain, body weight, a clinical score, burrowing behavior and nesting activity, mouse grimace scale (MGS), and drinking volume, were continuously monitored throughout the experiment. Blood and tissue samples were collected two weeks after BDL for further analysis.

Mice treated with metamizole and tramadol had a higher survival rate (70%) compared to carprofen-treated mice (30%). Distress score, body weight, and MGS increased significantly after transmitter implantation and BDL. While these parameters decreased rapidly after the implantation of telemetric devices, no recovery was observed after BDL. Across analgesic groups, no major differences were found in distress reduction. However, higher heart rate variability (SDNN) in tramadol-treated mice indicates a more effective pain relief in comparison to administration with metamizole and carprofen.

These results suggest that tramadol might be the most effective analgesic among those tested for the BDL model.

PB033

Assessing Competence, Cultivating Care: A Portuguese Collaborative Approach

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Abstract
Ensuring compliance with Ethics, Regulatory Directives, and National Legislation is imperative when using animals for scientific purposes. This commitment safeguards the well-being of the animals involved while promoting reliability and a good research outcome.

In this context, people are required to undergo a thorough process of education, training and supervision, culminating in the assessment of their competence in routinely performed techniques. Such a process is expected to be clearly defined, reliable, and consistent across trainers, assessors, and institutions.

Based on the EU Common Education and Training Framework document, a comprehensive methodology for Competence Assessment was developed by a working group representing four Portuguese research institutions.

This methodology includes detailed tabulated DOPS (Direct Observation of Procedural Skills) for common techniques involving laboratory rodents, addressing learning outcomes related to skills, professional attitude, knowledge, and the 3Rs. Clear guidelines and assessment criteria were also defined, ensuring consistency and standardization of this procedure across these institutions.

After two years of Competence Assessment implementation, this procedure has been considered remarkably positive, leading to significant improvements in the proficiency of those involved in the use and care of mice and rats for scientific purposes. It has also fostered a strong culture of care, and promoted mutual support and collaboration among researchers.

Equally important is that it also facilitates the free movement of personnel between institutions, with equivalent standards of competence.

We consider this methodology to be a reliable tool for skill validation, offering a model for harmonizing Competence Assessment in other institutions.

PB034

Capillary Micro Sampling in Mice and Rats

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Abstract
In regulatory toxicology studies, blood samples in rodents are usually sampled at the first day of dosing and again during the last days of the study. Blood samples are either sampled for clinical pathology analysis or to assess exposure to the test item, using bioanalysis. A standard is to include satellite animals for the sole purpose of drawing blood for bioanalysis and usually each animal will only be sampled a few times to ensure the sample volume stays within good practice guidance. In studies using mice and rats we included capillary micro sampling and eliminated the need for inclusion of satellite animals. Using capillary micro sampling we successfully collected up to 6 samples within 24 hours from each animal. In mice the sample volume was 20 uL and in rat the sample volume was 40 uL. Challenges with capillary micro sampling can be development of a bioanalytical method sensitive enough to handle small volume of blood/plasma as well as the need for additional handling of the capillary tubes following sampling to ensure stabilization of the sample in e.g. micronic tubes. The benefits are that fewer animals will be used in the studies; the full profile is obtained from a single animal reducing variability and increasing quality. Further, the small sample volume can more easily be collected and requires a shorter fixation period of the animal increasing overall animal welfare.

PB035

From IVF to Natural Mating in *Xenopus laevis*: A 3Rs Application

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Abstract
Xenopus embryos are usually obtained by in vitro fertilization (IVF) which allows getting embryos homogenous as stage but implies the killing of the males and the repeated handling of the females1. Thus, the natural mating (NM) effectiveness to substitute the IVF has been assessed to comply with the 3Rs. In fact, the natural mating (NM)[2] is a less stressful procedure and it doesn't require the male killing.

NM was introduced in our facility in October 2022 and results in terms of embryo quantity and quality were compared with the results obtained by IVF in the period 2017–2022.

NM resulted in an increase of embryos production and embryo quality has been comparable with the one obtained with IVF. The increase in embryo quantity has allowed to stimulate fewer females each fertilization round. Moreover, since male surgery and killing have been no longer necessary and they can be resued in mating, there has been a sensible decrease in the number of males used.

Furthermore, NM allows to obtain embryos homogenous as stage and at the beginning of the development for microinjection if embryos are collected at regular intervals. Embryos collection frequency can be adjusted according to the temperature and the needs of each laboratory.

NM resulted as a valid alternative to IVF and it complies both with Reduction, since less animals are needed, and with Refinement, since the procedure have an overall lower impact on animal welfare.

PB036

Environmental Health Monitoring in Mouse Facilities: Survey on the Status Quo in German-speaking Countries

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Abstract
The health status of laboratory animals plays a decisive role not only for the health and welfare of the animals but also for the validity of the study results. In recent years, there has been an increasing number of publications on environmental health monitoring (EHM). Molecular biological methods are used to detect nucleic acids of infectious agents in individually ventilated cage (IVC) systems, e.g. in exhaust air dust. This can reduce the number of mice used for health monitoring (HM) in conformity with the 3Rs. Numerous studies have shown that EHM is reliable and sensitive and is therefore a useful method for HM of mice. In order to assess the prevalence of the use of EHM in Germany, Austria and Switzerland and to better understand the factors that influence its use in facilities, a survey containing 33 questions was conducted by the Committee for Hygiene of the GV-SOLAS.

The survey revealed that 59% of facilities (91 in total) equipped primarily with IVC systems already use EHM to varying degrees, replacing between 4 and 2500 animals/year/facility. Beliefs on factors such as cost, time investment, and reliability of the methodology differ significantly between facilities that mainly use either animals or EHM. Additionally, the choice of monitoring strategy is influenced by the existing cage system and the availability of a decontamination option. The survey showed that there is still a need for information and a desire for further training on the topic of EHM.

On behalf of the Committee for Hygiene, GV-SOLAS

PB037

Neuro Score – An Added Informative Value for Severity Assessment in Neurological Rodent Models?

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Abstract
The legally required severity assessment of experimental animals, mandated by Directive 2010/63/EU, is typically conducted through daily visual inspection using clinical score sheets. Neurofunctional test batteries, originating from Samuel Irwin's 1968 protocol, are widely applied in preclinical research to assess side effects of drug candidates on the nervous system. Within the research consortium DFG-2591 the original protocol was modified to capture the severity of different neurological rodent models. This study aims at evaluating the added value of the *Neuro Score* compared to traditional clinical scoring.

We retrospectively analyzed and visualized the sum Neuro Score and clinical score of different neurological models along with single parameters categorized into four subdomains. The sensitivity of the *Neuro Score* in detecting changes in severity compared to the clinical score was statistically evaluated using a *two-tailed Fisher's Exact Test*.

Findings from the intrahippocampal kainate model reveal alterations in several *Neuro Score* parameters including changes in body tone, hyperlocomotion, increased reaction to sound and touch. While having the same sensitivity in detecting severity-associated alterations on the first day after induction, the *Neuro Score* proved to be more sensitive in chronic phases of the model.

Preliminary results suggest that the *Neuro Score* provides an added informative value compared to the traditional clinical score, particularly during the chronic phase. As single *Neuro Score* parameters allow for the detection of model-specific alterations with high granularity, incorporating them into model-tailored score sheets could improve severity assessment aligning with the 3R principle.

Supported by Deutsche Forschungsgemeinschaft (FOR2591, PO681/9-2 and-3).

PB038

Severity Heat Maps - Developing a Consensus on Cumulative Severity

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Abstract
Assessing actual severity helps us to understand the impacts on each animal undergoing procedures for scientific purposes, and also informs our plans for future work, our discussions on harms and benefits, and spurs us to implement refinements. Existing guidance on actual severity helps to benchmark our assessments and ensure they are performed consistently, but increasingly we are aiming to assess the cumulative impact on animals and need guidance on how to do this. We formed an internal working group comprising 14 people including animal technologists, welfare officers, vets, research scientists and statisticians who worked together to come to a consensus on how common clinical signs, including their number, magnitude and duration, affect actual severity for common laboratory species. The guidance is presented in a visual way, as "Severity Heat Maps", and in this presentation we will share how they were formed, how we use them and our plans for next steps. All animal studies are ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare, and Treatment of Animals.

PB039

Prediction of Germline Transmission Using STR Analysis Achieves an Enormous Reduction in Animal Numbers

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Abstract
Mutations are first specifically integrated into mouse embryonic stem cells (mES cells). Once the desired mutation has been confirmed in the mES cells, chimeric offspring are generated from these stem cells by microinjection into early wild-type mouse embryos. Since the chimeric progeny are mosaics, the success of the projects depends on whether the mES cells in the organism were also involved in the development of the germ cells. Frequently, no germline transmission occurs during mating. Nevertheless, a high breeding effort is required for this finding. This method contradicts the 3Rs (Reduction, Replacement, Refinement), which must be observed in experimental science.

In 2018, we were able to show that exact genomic differentiation of highly related mouse lines is possible using STRs (short tandem repeats). This technique has been successfully established in the analysis of chimeric sperm samples. With the help of STR analysis of sperm samples, the uncertain mating of chimeric males no longer has to be carried out.

The results of the analyzed chimeras lead to a candidate whose cryopreserved sperm is used for IVF, which specifically leads to a heterozygous F1 generation.

The STR analysis of the chimeras provided surprising results on the previously unknown distribution of ES cell-derived tissue in different organs.

The reduction of experimental animals through this technique is enormous and offers a major contribution in terms of the 3Rs. In addition to the effect that not all chimeric males are mated, many negative offspring are not produced in the first place.

PB040

Targeting Acral Melanoma in Mice by Voluntary Oral Delivery of Drugs

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Abstract
This project aims to advance animal welfare by introducing micropipette-guided drug administration (MDA) as an alternative to oral gavage for delivering drugs in the treatment of cancer. We applied MDA in a human melanoma xenograft mouse model and successfully administered kinase inhibitor.

Many cancer drugs are administered orally, and their development typically involves rodent models using oral gavage. However, oral gavage is stressful for both the animals and the personnel performing the procedure, and it carries risks of damage to the upper gastrointestinal tract.

MDA offers a non-invasive, stress-free, and precise drug administration technique, potentially overcoming the limitations associated with oral gavage. The study compares the anti-tumor response following the administration of small molecule cancer drugs via MDA or oral gavage. Our findings demonstrate that MDA is equally effective as traditional oral gavage in delivering cancer drugs, such as the tyrosine kinase inhibitor Regorafenib, and results in significant tumor regression following treatment.

This MDA project is part of a broader initiative aimed at discovering new drugs for the treatment of metastatic melanoma that remains a major health concern with limited treatment options, especially for patients without targetable tumour mutations. While kinase inhibitors have improved outcomes for specific patient subgroups harboring mutations in the cKIT receptor, effective delivery methods remain critical for advancing preclinical research. These findings suggest that researchers should consider substituting oral gavage with MDA, as it offers a more refined approach and minimizes stress for the animals.

PB041

A Comprehensive Welfare Scoring System for Graft Versus Host Disease in Humanised Mouse Models

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Abstract
Due to the limitations of using murine surrogate tools and differences in the immune system between mouse and human, immuno-oncology drug discovery is increasingly relying on humanised mouse models. Graft-versus-Host Disease (GvHD) remains a significant complication following allogeneic transplantation of human immune cells, which restricts the lifespan of mice as symptoms can become severe. Assessment of GvHD in these models is often hampered by the lack of a standardised scoring system. We have therefore created a comprehensive scoring system for GvHD in humanised mice. Offering a more precise and sensitive tool for assessing GvHD, our system helps minimise animal suffering through early detection and ensures interventions are timely and appropriate. This tool also supports the 3Rs by enabling mice to remain on study to achieve maximum scientific benefit. We have shown that the phenotypical scoring system correlates with pathological GvHD scores across multiple tissues. Liver and kidney pathology ranked highly with phenotypical score, whereas the lung did not. Furthermore, we have shown that the scoring system is reproducible, whereby independent experimenters score animals the same or very similar scores. Overall, the scoring system simplifies and standardises the decision-making process for animal technicians working across all our models, acts as a training aid for new technical staff, and supports our responsibility to minimise pain, suffering, distress, or lasting harm when working with animals.

PB042

The Adverse Effects of Adverse Affects

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Abstract
In most animal research facilities, there are times when a Standard Condition 18 report must be submitted if the severity limits set by the license or other controls are breached. Such breaches can occur, for example, when a new transgenic line displays an unanticipated phenotype not covered in the PPL or when an unexpected death occurs due to a scientific procedure. While adverse effects are common in research, they are rarely reported in publications. The ARRIVE Guidelines 2.0 recommend discussing the scientific implications of studies, including adverse effects, though this is not part of the essential areas to be covered in publications. Sharing unexpected adverse events within the research community presents an opportunity to refine methods, reduce animal usage, and minimize potential pain, distress, and harm. This poster will explore how research facilities can foster a culture of sharing these unexpected adverse events, offering insights into how they were overcome to achieve the scientific objectives of the research.

PB043

Ex Vivo Cavity Defect Model as Replacement of Short-term In Vivo Models

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Abstract
As scientists performing translational research with animals, our primary responsibility is to minimize and, whenever possible, prevent excessive pain and distress in research animals. One way to achieve this is by developing an ex vivo model that can reliably substitute for an animal model. Our aim was to create such a model to test the effectiveness of novel interventions in closing a subcutaneous cavity defect without the need for live animals. Subcutaneous fluid accumulation is a common complication that causes serious morbidity and delayed recovery.

We developed a model using ovine tissue from surplus animals euthanized for another experiment. Cavity defects were created by making a cranial-caudal incision in the skin and removing the underlying fat tissue. The pressure from surrounding tissue resulted in the formation of an oval defect. We measured the force required to approximate the defect along the incision midline using a pressure gauge. The defects were categorized based on the cumulative closing force and assessed for clinical relevance by a surgeon experienced in subcutaneous cavity closures.

We successfully created 22 defects per ovine cadaver. The cumulative closing force ranged 9 – 16 N, with forces below 9 N considered insufficiently challenging and those above 16 N deemed excessively challenging. The cumulative closing force could be increased by removing additional tissue with a thickness of 0.5 cm. These findings demonstrate the potential of our ovine cadaver cavity defect model as a robust alternative for performance testing of interventions for closing subcutaneous cavities without causing harm to living animals.

PB044

Multimodal Wound Healingplace Matrix in Wound Healing: Study Design to Reduce the Animals' Number

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Abstract

Purpose: The present study aimed to evaluate the wound healing process in rats, comparing untreated wounds to those treated with a multimodal wound healing matrix (Zenidic-MLM). By using each rat as its own control, we aimed to reduce the inter-individual variability and adhere to the 3R principles by minimizing the number of animals.

Methods: Two full-thickness excisional wounds (around 10mm in diameter) were surgically created the dorsal side, one wound on each side of the midline ($n = 20$). Rats were used due to their size that permits the creation of two sufficiently large wounds for monitoring healing. One wound was left untreated as a control, while the other one was treated using Zenidic-MLM for three days. Wound healing process was monitored over time by measuring the wound size every second day and performing histological analysis at three time points post-surgery.

Results and Discussion: The wound healing process was faster (4–6 days) using Zenidic-MLM compared to the untreated wounds. No sign of infection was observed in both groups during the study period.

Using each rat as its own control, reduced the total number of animals. Furthermore, using this approach allowed us to minimize the inter-individual variability. Using each rat as their own control allowed us to have a better understanding of the treatment efficacy with minimum interfere of the animal variability and thus a Reduction of the number of animals.

Conclusion: Because of precise experimental design, we could accurately evaluate the effectiveness of Zenidic-MLM while reducing the number of animals used.

PB045

Pain Relief and Recovery: Comparison of Oral Analgesics for Telemeter Implantation in Mice

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Abstract

Telemetric devices enable precise measurement of clinically relevant changes in animal's physiology by allowing continuous monitoring of ECG, body core temperature, and locomotion in preclinical studies. The implantation of such devices itself constitutes a harmful surgical intervention and must be refined to minimize surgery-related pain, enable sufficient recovery, and prevent impact on subsequent study outcomes.

BALB/c mice were continuously treated with either metamizole (3 mg/ml), tramadol (1 mg/ml), or buprenorphine (0.1 mg/ml) administered via drinking water, starting 12 days prior to the implantation of the telemetric devices. Animal welfare was assessed non-invasively by monitoring body weight, distress score, liquid intake, burrowing behavior, nesting activity, perianal temperature, mouse grimace scale, fecal corticosterone metabolites, and by telemetric monitoring of ECG, body core temperature, and locomotion.

Following telemeter implantation, a lower stress response and an accelerated recovery were observed in buprenorphine-treated mice based on the welfare parameters distress score, body weight, liquid intake, body core temperature, and locomotion when compared to analgesic treatment with tramadol or metamizole. In addition, buprenorphine-treated mice gained weight, resulting in baseline levels exceeding body weight after a recovery period of four weeks and had a consistently higher liquid intake compared to metamizole- and tramadol-treated mice.

Therefore, buprenorphine proved to be more effective in facilitating the recovery of mice after telemetric device implantation than metamizole and tramadol by restoring, and partially improving initial conditions.

PB046

Severity Assessment in Rats with Intracranial Glioma Using Voluntary Wheel Running and Weight

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Abstract

Animal models of intracranial tumor formation are essential for the development and improvement of novel therapeutic approaches. Likewise, objective parameters for severity assessment and humane endpoint determination are fundamental for ethical and legal reasons. We evaluated voluntary wheel running (VWR) in addition to daily assessed body weight and clinical scoring after tumor cell injection and tumor resection, as well as humane endpoint determination as measure for severity.

Male BDIX rats were either single-housed ($n = 14$) in wheel-equipped cages or group-housed ($n = 6$) in three Makrolon type IV cages connected by tubes and equipped with running wheels, where the rats could be identified by a subcutaneously placed RFID chip. Under general anaesthesia, both groups received a stereotaxic injection of glioblastoma BT4Ca cells into the frontal cortex. After eight days, the tumor was microsurgically resected in the single-housed rat group. Body weight, clinical score and VWR were monitored daily until the humane endpoint criterion of sudden weight loss and deteriorated clinical score.

In single-housed rats, body weight was slightly but significantly reduced after the cell injection and tumor resection ($p < 0.05$), along with reduced VWR ($p < 0.05$), while clinical status remained unaffected. On the day of the humane endpoint, sudden weight loss and clinical deterioration were associated with almost no use of the running wheel ($p < 0.05$). Analysis of group-housed rats showed showed similar results.

VWR monitoring can be used for objective assessment of the severity in rat models of intracranial tumor formation, including the determination of humane endpoints in both individual and group housed animals.

PB047

A Refined Method for Fecal Matter Transplantation

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Abstract
Fecal matter transplantation (FMT) is an expanding field of research, with more animals being subjected to forced oral gavage. Oral gavage requires restraining, which is time-consuming for technicians and stressful, potentially harmful for the animals, and may bias research results. Previous research has successfully demonstrated oral administration of drugs through voluntary intake, with Nutella® being a favorable choice.

Therefore, we hypothesized that traditional FMT techniques may be refined by using Nutella® for administration, thereby improving animal welfare, reducing workload pressure, and increasing safety, while maintaining the same rate of microbiota engraftment.

We tested the microbiota engraftment success of mouse-to-mouse fecal matter transplantation in antibiotic-treated BALB/c mice using three methods: traditional oral gavage, delivery to the oral cavity by pipette administration, and voluntary intake of a Nutella®-inoculum mixture.

The results of this experiment are expected to be ready before the congress. However, a preliminary pilot study comparing the Nutella® method to the pipette method showed equal engraftment success, as differences in microbiota composition between the donor and each group were similar, and the bacterial makeup in both groups changed equally towards the donor composition. Additionally, the Nutella® method was subjectively observed to be faster, safer, and less stressful for both mice and technicians.

PB048

Refinement of a Porcine Model of Abdominal Aortic Aneurysm

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Abstract
Modeling abdominal aortic aneurysms (AAA) is challenging due to the disease's multifactorial nature and associated comorbidities. We refined an established porcine AAA model to improve standardization while prioritizing animal welfare.

AAA was induced in four Cardio Pigs (male, 10 months old) using systemic oral BAPN, intraoperative balloon dilatation, and intraluminal elastase-collagenase treatment. Key refinements included the use of adult Cardio Pigs instead of juvenile Landrace pigs to ensure mature immune systems, physiological relevance, and vascular integrity, as well as longitudinal sonography for non-invasive aortic diameter measurement. The BAPN dose was halved to reduce lymph flow and lymphocele risk, and retroperitoneal access replaced laparotomy to minimize trauma and risk of wound complications. Temporary instead of permanent vertebral artery occlusion reduced the risk of medullary ischemia and subsequent paresis, while an applied Cook sheath avoided caudal mesenteric artery ligation minimizing complications in its vascular territory. Integrity testing of clamped aortic segments prevented enzyme leakage-induced circulatory instability, and pressure-controlled intraaortic enzyme delivery ensured standardization. Mean arterial pressure was maintained above 65 mmHg to ensure adequate organ perfusion during surgery.

All animals recovered uneventfully within 3–4 days without complications such as hind limb paresis, lymphocele formation, or aortic rupture. Sonography revealed progressive aortic dilation, with the mean diameter reaching 171% of baseline by day 28, alongside typical AAA morphologic and histologic features.

These refinements reduced animal numbers and minimized perioperative complications while ensuring standardization demonstrating the importance of targeted adjustments in advancing research quality and promoting animal welfare.

PB049

Humane Endpoints in Regulatory Toxicology in Fishes

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Abstract
Mortality is a key cause of severe suffering, and there can be an increased risk of this in tests using fishes, such as in regulatory toxicology procedures within OECD 203 and OECD 210 testing guidelines. In November 2023, the RSPCA organised an in-person meeting as part of our 'Focus on Severe Suffering' initiative, on the application of humane endpoints in regulatory toxicology studies that use fishes, with the aim of identifying and sharing strategies to reduce and avoid severe suffering. The meeting also explored some of the challenges associated with this goal. Participants concluded there is currently insufficient guidance on identifying sub-lethal clinical signs, and applying humane endpoints, for fishes used in regulatory toxicology tests such as OECD 203 and 210. The development of standardised practices would not only improve animal welfare, but also help ensure data reliability and regulatory compliance.

As a result of the meeting, the RSPCA has produced a report with targeted recommendations for the wider scientific community, including regulators, scientists, animal technologists and unit managers, and training organisations. This poster summarises these recommendations, which aim to significantly reduce suffering in fishes used in toxicology research.

PB050

Unveiling the Differences: How Oral Administration Methods Shape Pharmacokinetic Outcomes

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Abstract
Micropipette-guided drug administration (MDA) is a non-invasive alternative to oral gavage, though its limitations have yet to be fully explored. This research evaluated the pharmacokinetic (PK) properties of compounds administered via MDA or oral gavage to determine method equivalency for use in pharmacology studies.

In a pilot study comparing these two methods we observed lower systemic drug exposure in MDA-treated animals compared to those treated with gavage, results which we followed up on here. In the current study, systemic and organ exposure were measured following a single low, middle, or high dose (doses: 0.5, 2.0, and 10.0 mg/kg; volume: 2.0 ml/kg) treatment in C57BL/6J mice (n = 6 per group) administered by either MDA or gavage. Reduced systemic exposure at middle and high doses in MDA-treated animals compared to gavage groups was confirmed, while Tmax and Thalf were the same. The low dose exhibited equivalent AUC but a shorter Tmax in the MDA-treated group.

MDA resulted in reduced exposure, however dose linearity was maintained, and measures of absorption and clearance were similar. Variation in exposure was also lower with MDA compared to gavage, which could increase effect size and reduce sample sizes/animal usage. The composition of the vehicle also significantly influenced these outcomes in our pilot study, an effect that remains unexplored. Future research should examine potential mechanisms behind these differences to better guide the application of MDA in pharmacology studies.

PB051

Multimodal Research Approach in Noninvasive Perinatal Hypoxia in Rats

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Abstract
This research presents a multimodal approach to investigate the effect of non-invasive short-term perinatal hypoxia on rat brains, which includes behavioral testing, histological, immunohistochemical, and magnetic resonance image analysis (MRI). In total, 24 Wistar Han (RccHan: WIST) at postnatal day 1 (P1, weighed, randomly divided into hypoxic or control groups, keeping both sexes represented equally) were subjected to hypoxic (8%O_2 in 92%N_2) or normal conditions (21%O_2 and 78%N_2) for two hours. Neurodevelopmental behavioral and reflex testing was performed serially on each rat from P3 to P14. Eight animals per experimental group were scanned using 7TMRI with several imaging modalities. All animals were euthanized at P15, and the brains were isolated for histological and immunohistochemical labeling. Behavioral tests for sensory-motor and somatosensory development showed significant downregulation in rooting reflex and vibrissa placing response in rats post hypoxia. At P5, hypoxic rats showed persistent asymmetric limb movement in the ambulation test, unlike controls that started to move symmetrically, but hypoxic rats scored higher in the surface righting test. The *in vivo* DTI MR shows substantial differences in diffusivity detected in the anterior cingulate cortex at bregma levels 2.28 and 1.28 mm. The immunohistochemical labeling findings show an upregulation of MAP2 and a downregulation of neurofilament H expression in the same region. Future research will include transcriptome analysis and comparative research modality with scientific data obtained in the human perinatal brain.

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PB052

Quality Checklist for an Entrusted Performance Assessment

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Abstract
The humane treatment of animals used in science entails, amongst other requirements, that they are used and cared for by competent staff. Establishing competency in procedures in which animals are involved, is a prerequisite for performing animal experiments independently as stated in several articles, but mainly article 25 of Directive 2010/63/EU. Staff licensed to work with laboratory animals are only allowed to work unsupervised when assessed as competent. The last phase of training is often done within the research institute. There the licensed (young) professional is being supervised and assessed by senior colleagues as competent. These assessments primarily take place outside the boundaries of educational institutes, which could mean that they are performed by colleagues with limited didactical or assessment training. To enhance the quality of competence assessment in practice, part of the competence assessor outcomes developed with aid of the European Committee focus on establishing quality of assessment. This can be done using a quality checklist for an entrusted performance assessment which has been developed within ETPLAS E-learning module on competence assessment. It aids in reviewing, evaluation and improving the validity, objectivity, reliability, condition and purpose of any competence assessment in practice as part of plan-do-check-act assessment cycle. The list is developed, based on experience, literature and with input of various experts. It is now ready for user testing. By using and

optimizing this checklist both supervisors and competence assessors will receive valuable tools for the improvement and harmonization of competence assessment within their facility.

PB053

Benefit of Doubt: Test Analgesia to Refute or Confirm Counteractivity with an Experimental Model

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Abstract
Experimental models can induce pain. In general, analgesia will be applied to minimize discomfort. In models in which immunological pathways are involved, analgesia can interfere with the readout parameters[1] and can be a reason not to use them. Non-steroidal anti-inflammatory drugs (NSAIDs) have anti-inflammatory effects due to their inhibition of cyclooxygenase enzymes (COX1 and/or 2). Additionally, opioids have been shown to affect immune function in different degrees, although the underlying pathway has not been elucidated yet. Buprenorphine should have the least effect on immune function.

The unwanted immunological effect can preclude the use of analgesia in experimental models. Here we present an approach in which one experimental 'pilot'-group was added to the experimental design, which received analgesia to confirm or refute the effect on the experimental results. We present the findings in two different models. In an anastomotic healing mouse model, buprenorphine could be used without affecting the healing of the intestine. In contrast, in a thioglycolate-elicited peritoneal macrophage model, buprenorphine blocked the recruitment of inflammatory macrophages to the peritoneum and therefore, buprenorphine was not compatible with this model.

Conclusion: in models in which it is assumed that analgesia will counteract the model, the addition of a pilot group which does receive analgesia can give a better insight on the true interference. This approach uses additional animals, which will experience discomfort too, but can reduce severity in future experimental animals. From an animal welfare aspect, scientific information ('show me') is preferred over claims ('trust me').

PB054

Blood Collection from the Brachial Vein in Göttingen Minipigs

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Abstract
Göttingen minipig is commonly used in safety assessment studies of new therapies. While clinical pathology and toxicokinetic evaluation are usual endpoints in these studies, blood collection remains challenging in this species as most peripheral vessels are not readily accessible and other such as ear veins are not adequate for significant blood volume. At our site, the jugular vein was the default blood collection route but it has the following drawbacks: it is deep and not visible so there is a substantial risk of severe complications such as tracheal lesions or hemorrhage. Our objective was therefore to develop a safer alternative to the jugular vein for significant and repeated blood volume collection. The mammary vein and brachial vein were selected as potential leads for this objective.

Trials were conducted on colony animals to define the appropriate handling method, the anatomic markers and to confirm that the quality of the blood samples was satisfactory (no hemolysis nor clots). The mammary vein was less visible, induced frequent hematomas and was therefore not considered suitable for repeated samplings. Conversely, the brachial vein was well visible and hemostasis was easier to achieve. In a subsequent internal study, we validated that 0.5 ml blood samples can be collected 8 times over 24 hours on the 2 brachial veins by rotation, using an epicranial needle.

Following training of the technicians, blood collection from the brachial vein is now commonly used in our toxicology studies, reducing the risk of post-blood collection local effects and animal welfare events.

PB055

Mouse Model of Mild/Moderate DNCB-induced Atopic Dermatitis as a Tool for Preclinical Drug Testing

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Abstract
Atopic dermatitis (AD) is a prevalent inflammatory skin condition characterized by dryness, pruritus, and impaired skin barrier function. The condition is multifactorial in nature, involving diverse phenotypes and endotypes, which necessitates personalized treatment approaches. Current one-fits-all-therapies, such as corticosteroids, have limitations and side effects. In order to advance preclinical research in this area, a murine model was developed using 2,4-Dinitrochlorobenzene (DNCB), simulating mild-to-moderate AD and classified by comprehensive phenotypic and endotypic characteristics.

Our model revealed varying AD severities in female BALB/cJRj mice which underwent sensitization and daily DNCB induction over time. By day 12, moderate AD characteristics resembling subacute human AD, including hyperplasia, spongiosis, and a Type-1 immune response, were observed. By day 22, milder AD features akin to chronic phases with a Type-2 immune response emerged. Filaggrin expression correlated with disease severity, highlighting its significance in AD pathophysiology.

This mild-to-moderate model minimizes animal suffering compared to severe models, which are common but impose significant stress. Our comprehensive characterization bridges murine and

human AD subtypes, addressing gaps in the literature where inconsistent protocols hinder reproducibility. Immunological activation is confirmed, making this model suitable for drug testing without excessive disease severity.

Thus, our study aligns closely with the 3R principles by offering refinement through reduced animal distress, reduction by lowering the number of animals needed, and fostering replacement indirectly by improving model reproducibility, which may reduce reliance on in vivo experiments in the long term. This balanced approach enhances ethical considerations while maintaining scientific rigor in preclinical AD research.

PB056

Severity Assessment in Two Experimental Models of Subarachnoid Hemorrhage in Rats

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Abstract

Subarachnoid hemorrhage (SAH) is a severe neurological condition frequently studied using animal models to investigate its underlying mechanisms and develop new treatment options. The objective of this study is to assess the severity caused by two commonly used experimental SAH models in rats, in alignment with EU Directive 2010/63 ethical requirements for animal welfare.

The study was conducted on 140 male Wistar rats and compared two models of experimental subarachnoid hemorrhage: the injection model, which involves injecting autologous blood into the cisterna magna, and the filament model, which mechanically disrupts a cerebral vessel. To verify the occurrence of SAH, intracranial pressure and cerebral blood flow were monitored. The severity was evaluated through behavioral tests, including open field and burrowing activity, a neurological assessment using neuroscore, and the measurement of stress markers such as fecal corticosterone metabolites. Principal component analysis was utilized to identify sensitive indicators of distress.

The findings indicated that both models resulted in comparable levels of postoperative distress, as evidenced by similar severity grades. Significant behavioral changes, particularly a reduction in activity levels, were observed in both models. The results demonstrated that activity tests are highly sensitive tools for detecting distress and assessing animal welfare in models of acute cerebrovascular diseases.

This study highlights the necessity of selecting appropriate SAH models that balance scientific validity with ethical considerations. Both models caused similar levels of distress, indicating their potential interchangeability depending on research goals. Activity-based assessments provide robust data, offering a valuable addition to severity evaluation protocols.

PB058

The Latest Pathological Background Data Analysis of Rash2 Mice over a 26-week Experimental Period

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Abstract

CByB6F1-Tg(HRAS)2Jic (rasH2) mice are genetically engineered and commonly used in 26-week carcinogenicity studies, adhering to the ICH S1B guideline on testing for the carcinogenicity of pharmaceuticals. The rasH2-Tg(tg/wt) mice contain the c-Ha-ras (HRAS) gene. This strain is produced by cross-breeding C57BL/

6JJic-Tg(HRAS)2Jic (B6-Tg) hemizygous males and BALB/cByJJic (BALB) females. Here, we investigated the most recent background data of rasH2-Tg and rasH2-Wt mice. We examined 231 male and 230 female 34–36-week-old rasH2-Tg mice (tested for 26 weeks, starting at 8–10 weeks old), 50 male and 50 female rasH2-Wt mice of the same age, 50 male breeding retired B6-Tg mice (51–60 weeks old), and 50 female retired BALB mice (36–42 weeks old). Body weight was lower for rasH2-Tg mice than for rasH2-Wt mice (male, rasH2-Tg; 33.8 g vs. rasH2-Wt; 40.2 g; female, rasH2-Tg; 27.6 g vs. rasH2-Wt; 30.2 g). The survival rate of rasH2-Wt mice was 100% for males and females, 93.9% for rasH2-Tg males, and 96.1% for rasH2-Tg females. The most common spontaneous tumors in rasH2-Tg mice were bronchiolo-alveolar adenoma and hemangiosarcoma. The incidence of other tumors was extremely low. Accordingly, our recent pathological control data showed a low incidence of spontaneous tumors and a high survival rate in rasH2 mice. Thus, this study showed no significant phenotypic changes from previously reported findings, instilling confidence in conducting 26-week carcinogenicity studies. Furthermore, this prospective and longitudinal study demonstrated the animal model's robustness and minimal drift.

PB059

Do Animals Used in Research in Poland Suffer More Than in Other European Countries?

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Abstract
pt?>In 2022, Poland ranked 12th among EU countries in the number of animals used for research and education, amounting to 121,788. Alarmingly, 33% of these animals underwent severe procedures, significantly above the EU average of 11%, placing Poland in the second-highest position for this category. This trend has been consistent, with an average of 36% severe procedures between 2018 and 2021.

Our analysis suggests this high percentage may stem from the type of research conducted in Poland, particularly in neuroscience, where procedures tend to be more invasive. In 2022, 49% of all severe procedures were associated with basic research on the nervous system, and within this category, 46% were classified as severe—compared to EU averages of 12% and 8%, respectively. Additionally, 41% of procedures involving mice in Poland were severe (46% on average from 2018 to 2021), versus only 10% in the EU.

Another possible explanation lies in how severity is classified and reported. In the EU, severity is assigned prospectively (before experiments begin) and then reported based on actual outcomes. Differences in classification criteria or more cautious assessments by Polish Local Ethics Committees may contribute to these discrepancies. Polish researchers may also apply a stricter interpretation of severity criteria when reporting actual outcomes, further influencing the high percentage of severe classifications.

While the explanation likely lies at the intersection of these factors, a more harmonized approach to severity assessment and reporting across the EU could help eliminate or reduce these disparities.

PC001

Large Scale Eradication and Biocontainment of *Corinebacterium bovis* in a Rodent Facility

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Abstract
The objective of the present work is to describe the epidemiologic strategy as well as the operational and technical set of actions deployed at the Vall d'Hebron Institute of Oncology (VHIO) and aimed to eradicate *Corinebacterium bovis* from its rodent facility, with limited disruption of the daily operations and the scientific deliverables. *C. bovis* is a Gram-positive bacillus that causes a bacterial skin disease in immunosuppressed mice (hyperkeratosis). Transmission occurs between animals by direct contact or via fomites and it is well known for being the most prevalent contaminant of biological material such as PDX and cell lines used in preclinical oncology institutions such as VHIO, and for being extremely resistant in the environment despite the standard cleaning and disinfection routines. Infection in mice leads to lifelong skin colonisation and decreased engraftment success in patient-derived xenograft (PDX) tumour models. As a result of this any facility affected by C. bovis has a huge impact not only on the health and welfare of immmunodeficient mice, but also on the preclinical oncology study deliverables and on the overall operations, such as animal, materials and personnel flows.

VHIO underwent a two year global strategy that lead to a route map with active participation of the animal facility staff and the researchers.This thorough effort ranging from specimen decontamination up to operational routines redefinition resulted in the facility being declared free of C. bovis after two years and has remained so up to this date.

PC002

Refined Post-anesthetic Recovery of Göttingen Minipigs

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Abstract
Anesthesia is commonly employed in Göttingen Minipigs undergoing surgery, or other interventions not suited for awake animals. To various degrees, minipig physiologic- and hemodynamic parameters are affected by the anesthetic protocol chosen. Post-anesthetic care is important in ensuring smooth and rapid recovery and avoiding post-procedural complications. We describe four feasible methods of providing non-pharmacological post-anesthetic support: calm and safe housing, monitoring of vital parameters, supplemental heat, and soft padding for physical protection. The increased care regimen during recovery continues until the patient is alert, ambulatory, has swallowing reflex, and with satisfactory cardiovascular and pulmonary function.

The methods proposed are not exhaustive and measures such as preemptive and adequate analgesia as well as post-anesthetic welfare assessments are essential for optimal recovery.

PC003

Classification of Lesions in Subcutaneous Tumor Models in Mice

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Abstract
The Swiss Animal Welfare Officer Network (AWO-N) conducted a comprehensive survey in 2022–2023 to evaluate how subcutaneous tumor models in mice are managed across Swiss research institutions. The survey identified significant inconsistencies in the classification and assessment of skin lesions, necrosis, and ulceration. Researchers reported difficulties and inconsistencies in defining these critical areas, highlighting the need for standardized classification methods.

In response, the AWO-N developed the poster "Classification of Lesions in Subcutaneous Tumor Models in Mice." This poster is designed for display in research facilities and serves as a visual guide to evaluate skin lesions and identify early signs of necrosis and ulceration in subcutaneous tumors.

The goal is to establish a standardized approach to lesion assessment, ensuring more consistent and reliable evaluations across different research settings.

While the poster does not include specific humane endpoints, it aims to reduce ambiguity and enhance clarity in the classification of skin lesions.

This initiative is expected to improve research quality and animal welfare by promoting consistent practices and fostering a shared understanding among researchers, animal welfare officers, and authorities. Through these efforts, the AWO-N aims to address existing gaps and achieve a broader consensus in the field.

PC004

Cortisol Measurements in Different Mouse Strains during Construction Work in a Laboratory Animal Facility

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Abstract
Stress related health problems are a major restriction in the welfare of lab animals. There are numerous studies regarding stress signs and welfare problems caused by high noise levels and ambient vibrations. The aim of our study was the analysis of the stress level of mice in a laboratory animal facility during construction work compared to quiet surroundings. The cortisol level of 120 cages with 2 female mice per cage of 12 different mouse strains and additional 50 cages with 1 male mouse was analysed. The results showed significantly different cortisol levels during construction times compared to quiet surroundings in most mice strains. Our work shows the possible influence of loud noise and vibrations on the stress level of laboratory rodents and could perhaps be used as a reference point for stress reduction during construction work in the future.

PC005

Descriptive Study of Clinical Signs in Laboratory Mice in an Experimental and Breeding Facility

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Abstract
INTRODUCTION: Proper identification of clinical signs ensures compliance with current legislation and contributes to animal welfare evaluation and health monitoring.
AIMS: Quantify and categorize the most common clinical findings in a rodent facility.
METHODOLOGY: Clinical signs from mice reported to the veterinary area of Barcelona Science Park during 2019 were analyzed. All animals were clinically inspected by board-certified veterinarians.
RESULTS: 1321 clinical signs were documented. The age at clinical inspection was 10–28 days (44 cases), 1–6 months (701 cases), 6–12 months (360 cases), 12–24 months (141 cases), ≥24 months (62 cases), not determined (13 cases). Sex distribution: 672 females; 624 males; 25 pre-weanling animals. Most predominant genetic backgrounds were C57BL/6 (835/1321) and FVB (193/1321). The most common clinical signs reported were subcutaneous tumors (310/1321); ulcerative wounds related to fighting (178/1321); ocular signs (123/1321); non-specific clinical signs related to poor condition – i.e. cachexia, lethargy, poor coat condition, kyphosis - (122/1321); neurological signs (111/1321) rectal

prolapse (109/1321) and ulcerative dermatitis (99/1321). Other less reported included obstetric signs (46/1321); malocclusion (45/1321); runted pups (39/1321); different signs in the tail (25/1321); hydrocephalus (24/1321); other lesions in the skin (21/1321) and signs located in the penis (19/1321) and vagina (14/1321). Clinical signs of poor condition were mainly attributed to experimental conditions/phenotype (65/211) or spontaneous disease (18/122). Subcutaneous tumors were mainly attributed to phenotype (190/310) or experimental conditions (65/310).

CONCLUSIONS: Personnel involved in animal research must be aware of distribution and presentation of clinical signs according to the features of each facility, strains and experimental procedures.

PC006

Considerations for Improving Animal Husbandry Practices for Laboratory Rodents

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Abstract
While animal husbandry practices have come a long way in terms of improving care and welfare of laboratory animals, additional research remains necessary. Unfortunately, technology has not yet reached the point where the use of animals in research can be completely avoided, thus, it is our ethical responsibility as researchers to provide the best care possible. To gain a better understanding of optimal husbandry practices, the impact of various animal handling protocols, cage enrichment strategies and aberrant ultrasonic noise on rodent behaviour and physiology has been investigated. Both differences in experimenter familiarization/handling practices and cage enrichment were shown to impart long-lasting changes on rodent behavior and physiology signifying the importance of optimizing practices in favor of animal welfare. In addition, the presence of ultrasonic noise within laboratory facilities represents an often-overlooked animal husbandry consideration as such noise cannot be detected by humans without specialized equipment. Given the extended hearing capacity of laboratory rodents, ultrasonic noise represents a variable that should be controlled within the animal environment similarly to how other environmental factors including temperature, humidity, light, and audible sound, among others, are controlled. Assessment of the impacts of ultrasonic noise on rats yielded significant differences in behavior and physiology proving the importance of considering such hidden factors in animal husbandry protocols. Overall, the optimization and increased reporting of such practices within the literature will serve to improve animal welfare by raising awareness of the myriad contributing factors as well as mitigate issues of reproducibility within animal research.

PC007

Effects of Enriched Housing on the Behavior and Physiology of Laboratory Rodents

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Abstract
Environmental enrichment in rodent research has garnered increasing attention and scrutiny in recent years; however, it is important to keep in mind that not all enrichment scenarios are beneficial. Understanding the impacts of various enrichment scenarios is complicated by factors such as sex and age. Conventional rodent housing consists of shoe-box style cages provisioned with some form of enrichment but enriched cages featuring increased vertical height with the addition of a shelf are becoming more popular. To gain an understanding of the effects of such modifications, behaviour and physiology of adult male and female rats housed in standard versus enriched cages was assessed. Rats housed in enriched cages showed significant differences in behaviour and physiology compared to those housed in standard cages although additional testing is necessary to elucidate whether enriched cages improve overall animal welfare. Additionally, given the unique needs of nursing dams, the effect of such housing modifications on dams and their offspring was also assessed. It was expected that cages featuring a shelf would improve welfare given the ability of the dam to escape the demands of her pups – a situation reflective of natural rodent behavior. Results indicated that rats reared in enriched cages had lower body weights, higher fecal corticosterone and increased anxiety-like behavior suggesting that the ability of the dam to escape her pups as frequently as she chooses may not be beneficial to her offspring. A thorough understanding of the impacts of enriched cages will assist in enhancing overall welfare of laboratory rodents.

PC008

Comparative Analysis of Sperm Parameters between Different Mouse Strains

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Abstract
Wild-type mice are used to generate new genetically altered mouse models through transgenic methods. Understanding the genetic background is essential for evaluating fertility and reproductive performance in genetically altered lines. The aim of the

study was to investigate the sperm motility and morphology of three commonly used genetic strains: C57BL6/J, BALB/c, and CBA/J, utilizing advanced sperm analysis techniques. Using the Computer Assisted Sperm Analyzer (CASA) system, specifically the SCA software, we conducted a comprehensive assessment of sperm motility parameters, including kinetic metrics and types of motion. Additionally, morphometric characteristics of sperm were recorded following treatment with the SpermBlue® staining protocol. The protocols and methods for this study have been rigorously tested in our laboratory and several data for kinetic and morphometric values have been collected. A comparative analysis was performed among the three strains. Our results have revealed significant differences in the progressive motility and many of the morphometric parameters. The values of sperm parameters may provide a phenotypic platform for evaluating genetically altered mice, enhancing insights into their reproductive health and welfare. Furthermore, our study addresses the critical need for reliable sperm cryopreservation methods, as means of reduction, establishing a robust quality control system for sperm samples. The findings will contribute to the characterization of genetically altered mouse models and deepen our understanding of factors influencing sperm quality across different strains.

PC009

Transitioning from Traditional Water Bottles to Water Pouch Systems for Rodents

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Abstract
The Department of Comparative Medicine (AKM) at Karolinska University Hospital replaced its aging 15-year-old IWT Multi Magic bottle washer to address inefficiencies, labour-intensive workflows, and inconsistent water quality. This transition from bottles water pouches aimed to improve working conditions, reduce resource consumption, and enhance animal care practices. The objective of this study was to assess the impact of transitioning to the Avidity Science Sipper Sack System on working conditions, resource efficiency, and water quality in laboratory animal facilities. The implementation involved replacing traditional water bottles with sterile water bags fitted with reusable stainless steel drinking nozzles containing check valves. Metrics assessed included technician workload, water and energy consumption, space utilization, and water quality. Data were collected through workflow observations, utility usage logs, and microbiological analyses before and after the process. The transition eliminated bottle-handling tasks, reducing technician labour and monotony, significantly. Water and energy consumption decreased by approximately 80%, while space requirements for water management equipment dropped by 90%. Microbiological analyses confirmed consistent, high-quality water throughout use, showing no risk of contamination due to the sterile and sealed bag design. Avidity Science's Sipper Sack System offers a sustainable, efficient, and contamination-free solution for laboratory animal water management. This innovation improves technician workflows, reduces environmental impact, and ensures high water quality, demonstrating its value as a superior alternative to traditional bottle systems.

PC010

Benchmarking Technical Staff Ratios for Laboratory Mouse Care: Progress and Preliminary Survey Results

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Abstract
The care of laboratory animals, particularly mice, is crucial for scientific research and relies heavily on technical personnel. However, the staffing requirements vary significantly across facilities due to differences in housing conditions, husbandry practices, and technological advancements. To address this variability and provide guidance on appropriate staffing levels, our working group is undertaking a comprehensive benchmarking exercise. We developed two surveys to collect data on staff numbers across various specific conditions in different facilities.

The initial exploratory survey is designed to assess aspects of laboratory mouse facility management, including the mapping of staffing levels, staffing requirements and possible reasons for difficulties in meeting the needs for the continuous care of animals.

A more detailed second questionnaire is addressed to voluntary participants of the first study, focusing specifically on the labor requirements for operating a laboratory mouse facility, particularly at the level of animal caretakers, animal technicians and animal technologists1. This survey captures the workload associated with animal care and supporting activities, categorized by task types and various technical staff roles.

Preliminary results from both surveys are presented. Our analysis aims at providing benchmarking figures for appropriate staff numbers for laboratory mouse housing and care. The data generated from this study will enable facilities to compare their staffing levels, identify potential workforce gaps, and implement corrective measures where necessary. In addition, the results are intended to serve as a basis for future discussions on optimizing staffing levels in specialized mouse laboratory animal facilities.

PC011

Optimizing Pain Management Protocols: A Survey on Sustained-Release Buprenorphine in Laboratory Rodents

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Abstract
Buprenorphine, an opioid, is often used for post-surgical pain management in laboratory mice and rats. In recent years, buprenorphine has become available in a sustained-release formula.

Common methods of buprenorphine administration are subcutaneous injections 3–4 times daily or voluntary oral intake of a buprenorphine-Nutella mixture every 12 hours. Frequent injections put a strain on laboratory rodents and demand researchers' presence at unusual hours. Oral administration depends on voluntary consumption of the medicated Nutella. If the rodents do not consume the mixture, frequent injections become necessary.

To address these challenges, we obtained a permit to import and use Ethiqa XR, a sustained-release buprenorphine product. Ethiqa XR is formulated specifically for laboratory animals, with a documented effect for up to 72 hours in rodents, eliminating the need for frequent injections. A disadvantage of the lowered injection frequency is that the animals are potentially assessed for signs of pain fewer times daily. Early feedback on the product has been positive, although it is more expensive than short-acting buprenorphine. We hypothesize that its benefits outweigh the disadvantages.

We launched a survey to gather researchers' experiences with sustained-release buprenorphine, including its effects and any adverse reactions. We also aimed to evaluate if sustained-release buprenorphine use led to changes in welfare check frequency or distribution.

In conclusion, the survey investigates the perceived effect of sustained-release buprenorphine in laboratory rodents with the focus of refinement for rodents and improved working conditions for researchers. This insight will enhance our understanding of post-surgical management of rodents at our facility.

PC012

Implementation of Tunnel Handling at a Large Animal Facility

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Abstract
Tunnel handling reduces anxiety and stress in laboratory mice. To enhance animal welfare, we are implementing tunnel handling at our department with approximately 8000 cages housing laboratory mice cared for by 70 caretakers and students.

We planned the implementation in steps for a smooth transition. First, we selected a 100 mm long, clear acrylic tunnel for its suitability and visibility. We then conducted a pilot project in one animal unit to gain practical experience, assess time for cage changes and daily checks, and evaluate ease of washing and handling the tunnels.

In the near future, we plan to expand the use of tunnel handling across all animal units. This will involve tunnel handling ambassadors from each unit, who will attend a tunnel handling workshop where they can practice tunnel handling and understand the scientific background. We expect these ambassadors will play an important role in ensuring a smooth transition from tail to tunnel handling at their respective units.

Preliminary experiences from the caretakers are that after a brief introductory period, tunnel handling is not more time-consuming than the previously used tail handling method, but correct placement of the tunnel is important to avoid hindering correct closure of cage-lid. Experience has shown that mice are easier to familiarize with tunnel handling if the tunnel has been placed in the cage beforehand. Overall, the experience of the caretakers is that the mice appear calmer after becoming accustomed to tunnel handling. Experiences from implementation across units will follow at the conference.

PC013

Chlorine Levels In Drinking Water Impact Intestinal Flora of Guinea Pigs

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Abstract
BACKGROUND: Animal facilities often use automatic drinking water systems. The water is typically treated with chlorine to reduce the risk of bacterial contamination.
AIM Evaluate the impacts of chlorinated drinking water on the gut flora of guinea pigs (GP)
METHOD: 40 GP (24 young and 16 retired breeders) were used for this study; 10 were given water with 0.1 PPM chlorine and 30 were given water with 3 PPM chlorine) for 4, 5, and 6 months. Animals were euthanized and 1 gr of mixed intestinal content was collected and diluted in 20 ml sterile saline. Serial dilutions were plated in triplicate on two types of media and incubated at 37°C aerobically and anaerobically for 72 hours.
RESULTS: Results are expressed in colony forming unit/g (CFU/g) for aerobic, anaerobic, and coliform bacteria. No significant decrease in bacterial load was observed in either group. A significant increase ($P = 0.030$) in anaerobic bacteria was observed in the animals given water with high levels of chlorine. In addition, a significant increase in bacterial load ($P = 0.036$) was observed in old animals relative to young for all bacterial groups.
CONCLUSION: The use of highly chlorinated water does not cause a decrease in the bacterial load in the intestinal flora of guinea pigs. However, a statistically significant increase in bacterial load is observed between the treated and the control groups as well as between the young and old group. Interestingly, no clinical impacts were associated with this increase.

PC014

First time in Israel, the Rehoming of Gottingen Minipigs

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Abstract
Rehoming Göttingen Minipigs (GMPs) post-experimentation presents unique challenges due to sterilization regulations, behavioural needs, and public misconceptions. Successfully finding lifelong homes for these intelligent animals requires addressing welfare concerns, transportation logistics, and health compliance. Ethical considerations and public awareness are critical to ensure their reintegration into appropriate environments, especially in regions lacking local guidelines for such efforts.

MD Biosciences embarked on a journey to rehome three female GMPs with a successful outcome. The process began with identifying a farm open to accepting research animals, despite no prior experience with GMPs. Together, a comprehensive rehoming program was developed to address their dietary, enrichment, and acclimation needs.

Municipal approval was obtained for transportation between Rehovot and Ramat-Gan municipalities. The pigs were sterilized on the donor's site by veterinarians and allowed time to recover. Authorized transport was arranged, including familiar food and toys to minimize stress. Collaboration between donor and receiving veterinarians ensured a gradual dietary transition and consistent health monitoring. Emotional bonding was fostered by naming the animals, Reichel, Fibi and Monica, symbolizing their successful integration into their new environment.

The process raised important ethical questions: Who oversees the animals' health in their new homes? How long should donors remain involved? What protocols should be in place for negative human-animal interactions? And how should suitable homes be selected?

This journey underscores the importance of collaboration, adaptability, and ethical reflection, setting a precedent for future rehoming efforts and emphasizing the welfare of research animals post-study.

PC015

Bringing Experts Together for Successful Surgery: Working Well as a Team

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Abstract
If we refer to human surgery, surgical practice is clearly a team effort requiring a combination of diverse skills alongside rigorous organization, planning, and communication to ensure success.

In the context of animal experimentation, although numerous guidelines encourage researchers to surround themselves with the necessary expertise for their projects (e.g., PREPARE Guidelines), a quick assessment reveals that researchers often remain isolated, insufficiently trained, and poorly prepared when they initiate projects involving surgical procedures.

In many cases, they are not even aware they are performing surgery or unconsciously underestimate the complexity and impact of the procedures they conduct on live animals.

This issue highlights a critical gap in the integration of collaborative practices in preclinical research involving surgical models.

Using the specific example of developing a chronic porcine model for uterine transplantation, we will illustrate how individuals from diverse fields—including medical doctors, researchers, veterinarians, engineers, and technicians—can work collaboratively toward a shared objective. Each professional contributes unique and complementary skills that are crucial for the success of the project.

Through clear goal-setting, engaging training tools, and structured planning, we demonstrate how it is possible to build a motivated and effective multidisciplinary team. This networked collaboration enables the successful execution of complex surgical projects while ensuring that scientific objectives, refinement of techniques, and animal welfare are seamlessly integrated. Moreover, such an approach fosters the reproducibility and reliability of experimental results, underscoring the importance of teamwork in achieving ethical and high-quality research outcomes.

PC016

Exhaust Air Dust Testing Can Early Detect *Pneumocystis spp.* Infection – A Case Report

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Abstract
The individually ventilated caging (IVC) system has been widely used in rodent animal facilities in recent years. The soiled bedding sentinels (SBS) are commonly used for health surveillance in this system. However, not all pathogens can be transmitted through soiled bedding, such as *Pneumocystis* spp. The collection of exhaust air dust (EAD) of IVC racks followed by PCR analysis is another approach for health surveillance in the IVC system. Here, we report a case of *Pneumocystis* spp. infection that occurred in our animal facility, which we detected earlier using EAD compared to SBS testing.

All mice in this study were housed in the IVC system in the vivarium. The SBS system was used for routine health surveillance, while EAD testing was also performed as confirmation. The lung tissue of SBS was collected for the detection of *Pneumocystis* spp. by qPCR analysis. For EAD testing, dust particles were taken from pre-filter of an IVC air handling unit using swabs. The swabs were then submitted for DNA extraction followed by qPCR analysis.

In the health surveillance of first season 2024 in our facility none of SBS samples were tested for Pneumocystis spp. positive; however, 3 racks of EAD samples were positive. We then randomly checked colony mice in these racks and found that there were *Pneumocystis* spp. positive animals in all 3 racks. In conclusion, EAD method was more sensitive in detecting *Pneumocystis* spp.

Therefore, we suggest that EAD testing could be an alternative to SBS method for detection of *Pneumocystis* spp.

PC017

A Spontaneous Phex Mutation in C57BL/6JNarl Mice as a Model for X-linked Hypophosphatemic Rickets

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Abstract
Human X-linked hypophosphatemic rickets (XLH) is a rare disease affecting approximately 1 in 20,000 individuals, characterized by hypophosphatemia. Affected children exhibit slow growth, short stature, and bowed legs. In a C57BL/6JNarl massive production colony, a small number of weaning mice exhibited an abnormal gait characterized by slightly outward splaying of the limbs, smaller body size, and shortened limbs and tails, with a prevalence of approximately 0.001%. Necropsy examination revealed shortened and deformed femurs. Histopathological analysis of the femurs showed widened long bones, thickened growth plates, insufficient bone formation, and poor mineralization. Serum biochemistry analysis showed hypophosphatemia (5.65 ± 0.51 mg/dL) with normal calcium levels in these abnormal mice. Based on the observed phenotypic findings, these mice were diagnosed with rickets. In addition, a male affected mouse was subjected to a mating test to investigate hereditary transmission, which confirmed that the rickets phenotype is an X-linked genetic disorder. According to previous reports, the Phex gene was identified as the primary candidate gene that might be involved. A point deletion in exon 9 of Phex mRNA was detected, and this missense mutation resulted in substitution from R358K, associated with a truncated Phex peptide at position 366. A retrospective review of the breeding colony was conducted, and suspicious breeder mice were excluded. No additional rickets-affected mice have been observed in the colony for over a year. A pedigree of C57BL/6JNarl-Cg. $Phex^{NLAC}$ mutant mice was subsequently established as an animal model for XLH, representing the first spontaneous Phex nonsense mutant mouse with a point deletion.

PC018

Breaking Isolation: Loft Housing for Guinea Pigs in Research

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Abstract
The housing of guinea pigs in individually ventilated cages (IVCs) offers significant advantages in respiratory research, especially to maintain a controlled airflow, to improve hygiene, and standardize environmental conditions. By providing a controlled air exchange, IVCs minimize the spread of allergens, ensuring a safer environment for operators and reducing the risk of animal contamination from pathogens that is essential for animal welfare and research outcomes. Current legislation imposes strict requirements regarding the minimum space that each animal must have available. This can limit the ability to house an adequate number of subjects in standard IVCs, often leading to individual housing, with detrimental effects on the physical and psychological wellbeing of the animals. We therefore investigated an innovative solution for increasing cage surface area through the implementation of a loft, allowing for the housing of up to three subjects in a single cage. We designed a loft system that could be integrated into standard cages already in use. The loft consists of a single wide platform that provides additional space while ensuring the safety and comfort of the animals. We analysed behavioural indicators, physiological indicators, physical health indicators and operator feedback on functionality of the new loft cages.

Guineapigs housed in loft cages, exhibited increased exploratory activity and social interactions. The animals regularly utilized the platforms, showing interest and curiosity towards their environment and the additional space.

Our study demonstrated that the implementation of loft cages can significantly improve guineapig welfare by optimizing the use of available housing space.

PC019

Genetically Modified Colonies: Innovation to Enhance Management Processes

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Abstract
Over the years, Charles River has sought to have an integrated approach to the management of genetically modified colonies. This has been made possible through the development of Internet Colony Management *software* (ICM™) that takes into account all the processes involved in breeding.

ICM™ collects colony data through an RFID system placed on the isolators, IVC and cages, making it versatile and flexible depending on the *housing* system used. Colony data and activities performed will be easily visible on a tablet/PC/cell phone after they have been captured, ensuring 24-hour access to colonies status.

In conjunction with ICM™, Laboratory Testing Management (LTM™) has been developed, a [software] that makes it possible to request health checks, genotyping and then archiving of the data obtained as a result of analysis. Sanitary diagnostics are carried out using minimally invasive methods.

As a complement, we thought of an innovative approach with regard to the identification of the individual animal, having as objectives, not only the improvement of welfare, but also operational; this was possible thanks to the use of small earrings with a 10-colour scale and QR codes. Identification with these earrings,

together with the use of non-invasive sample collection techniques for genotyping, allowed for optimal integration of our systems, with benefits in work processes and data recording.

This integrated system will give evidence of how traditional practices, supported by computerized management and revised diagnostic methods, have contributed to improved colony management methods, with animal welfare as well as operational benefits.

PC020

Using Filters in the Sump for Monitoring Health of Laboratory Zebrafish

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Abstract
Zebrafish (*Danio rerio*) are useful in scientific research due to their close genetic similarity to the human genome and fast reproductive lifecycle. Their increased use in scientific research calls for improved methods of monitoring their health, as current methods involve multiple types of testing including submission of whole fish to identify various pathogens. This study developed a novel sampling technique by exposing nitrocellulose filters to sump water over 30, 60, or 90 days. The filter was compared against other known testing methods of swabbing biofilm from the sump, passing sump water through a vacuum filter, and whole fish PCR. It was hypothesized that the nitrocellulose filter would identify more pathogens over time, reducing the need for multiple testing methods. PCR testing was conducted to detect *Mycobacterium chelonae, Mycobacterium fortuitum*, Zebrafish picornavirus, *Myxidium streisingeri, Pseudocapillaria tomentosa, and Pseudoloma neurophilia* at each timepoint. *P. neurophilia and P. tomentosa* were not detected by any of the three environmental sampling techniques. Test filters were most sensitive at detecting *Mycobacteria* spp. and Z. picornavirus, while water filtration was most sensitive at identifying *M. streisingeri*. Swabs of the sump biofilm were highly variable in identifying pathogens. Testing the filters at 60 days yielded the highest pathogen detection. Nitrocellulose test filters may be a less labor-intensive method for health monitoring of laboratory zebrafish colonies at 60 days of sump exposure and may reduce the need for alternative pathogen detection methods.

PC021

Development of Monitoring Sheets for Sheep and Pigs Undergoing Cardiac Surgery

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Abstract
Correct follow-up of research animals is essential to refine experiments and reduce pain and suffering. Standardized sheets facilitate this process. Although the term score sheet is more often used, we have decided to use the term monitoring sheet as score sheets often imply a numerical scoring system which is not always easy to use. Besides, our primary aim was to define actions to take when a single parameter deviated, and not on a sum of scores.

To develop the monitoring sheets for pigs and sheep undergoing cardiac surgery the following people were involved: the caretakers, technicians and researchers closely involved in these experiments and the designated veterinarian. Together, we defined which clinical signs can be seen, how often these should be assessed and which action should be taken when a parameter deviates from normal.

The following parameters were included for pigs and sheep: food intake, general condition/activity, pain, bleeding, infection, breathing and oedema. For the pigs we also included cyanosis as this is sometimes seen in the model of myocardial infarction. As in sheep valves and cardiac assist devices are implanted, these are also included for the sheep.

These monitoring sheets were then presented to the Animal Welfare Body and subsequently approved by the institutional Ethical Committee. The monitoring sheets must now be used in all projects involving this type of experiments.

PC022

Evaluation of Retroorbital (Retrobulbar) Blood Sampling in Rodents

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Abstract
Blood collection is a common and important procedure used in animal experiments. There are several different methods to perform it, each with advantages and disadvantages. Regarding animal ethics, the blood collection methods that reduce pain or injury, and cause less stress in experimental animals are important. Various comparative studies of blood collection methods have been reported in the last two decades. Retroorbital or retrobulbar blood sampling is a common technique used in laboratory rodents, with many well-known pros and cons.

The aim of our study was to perform a literature survey that included original research, review articles, metanalysis, websites, and platforms in connection with animal experiments regarding retroorbital (retrobulbar) blood sampling.

PC023

Rabbit Medical Training: A Reasoned Approach and Creation of Educational Sheets

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Abstract
In light of a survey of pet rabbit owners, medical training appears to be a promising and interesting tool to prevent and counter the difficulties encountered during care or treatment administration. Its use is still poorly documented in pet rabbits but it showed benefits in many other species, wild or domestic. Its implementation is based on targeted learning mechanisms (positive reinforcement in particular) that will maintain the rabbit's motivation while strengthening the link with its owner. This work resulted in the creation of practical sheets describing medical training sessions in detail. This educational support was developed with the aim of providing users the objectives, the list of necessary equipment, the frequency and duration of the sessions as well as illustrated protocols detailed into simple steps. The use of these tools as well as the attention paid to the rabbit's needs and behavior should improve its well-being and health.

All the elements are detailed in the veterinary thesis defended by Mrs. Lena Pertuis, in October 2024.

PC024

Assessment of the Welfare of Domestic Horses According to the Housing Conditions

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Abstract
Animal welfare is an evolving concept and a major societal concern. The domestic horse is a species whose physiology has been studied at length and this scientific knowledge has made it possible to determine the optimal living conditions of this equine and to establish criteria for evaluating equine welfare. This study, focused on the well-being of horses in their domestic habitat, led to the creation of a welfare assessment grid. This tool, applied in the field, has not only highlighted the different specificities of the main existing accommodation methods, but also highlighted their strengths and weaknesses. The involvement of horse owners in this project, through the rating of their animal's level of well-being, has also made it possible to take stock of knowledge of equine welfare criteria, and highlighted the lesser-known criteria. Finally, the analysis of the results, in addition to revealing the difficulties encountered in applying these predefined criteria in the field, led to the development of avenues for improvement concerning the major problems encountered, whether they are common to the horses evaluated or related to their mode of accommodation. All the elements are detailed in the veterinary thesis defended by Mrs. Léonie Blanchard-Jouninet, in July 2022.

PC025

Regulatory Training "Initiation to Surgery on Fish Used for Scientific Purposes"

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Abstract
With 27.6% of animals used in Europe (2020) and 10.5% in France (2021), fish constitute the second category of species most used for scientific purposes.

Many laboratories (Ifremer, Inrae, Cirad, Universities, high schools, private industries, ...) use fish as a biomedical animal model or as sentinel in ecotoxicity studies or for population monitoring and biomeasurement recordings on wild populations.

In certain cases, it is necessary to realize surgery to implement measurement, identification or remote detection systems.

Oniris (Nantes Veterinary School) hosts a regulatory training module in fish experimental surgery (Training in good practices in anesthesia, pre, intra and post-operative analgesia, asepsis, sutures, holding instruments and application of end points) approved by the French Ministry of Agriculture since 2011.

Specific training in surgery for "Fish and amphibian" species in France was absent before 2011. Training set up was made on demand and with the help of the fish users concerned (students and trainees).

This training course is an answer to respond to regulations in addition to the regulatory animal training for personnel (scientists and technicians) using aquatic species (fish or amphibians) performing surgical procedures on live animals (laboratory, wild).

PC026

Digital Ventilated Cages for Data Acquisition of Animal Activity and Bedding Conditions in Mice

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Abstract
Digital ventilated cage (DVC) used for real-time monitoring of home cages and data acquisition through contactless electromagnetic fields of capacitance sensing technology positioned beneath the cage floor. The objectives are to generate data for animal

activity index (ALI), bedding status index (BSI) of dirtiness, regularity disruption index (RDI), Diurnality of animal activity at light phase and Frontality of mice. The activity was evaluated in three mice strains (ICR; C57BL/6 and BALB/c) with four bedding materials *viz* Alpha-Dri; Safe Pure; Corncob (medium) and Corncob (small). DVC system was validated for 8 consecutive cage changing cycles and data collected were analyzed from all the groups. The cage changing frequency was followed on 7-, 10- and 14-days interval where 5, 4, 3 or 2 mice were housed with enrichments, respectively. The results of ICR mice revealed that significant increase in activity followed by C57BL/6 and BALB/c including 24 h averaged smoothed data and regularity disruption of both light:dark phases. Alpha-Dri and Corncob (small) bedding exhibited lesser dirtiness on day 14 and Diurnality showed higher in ICR mice than other two strains. Frontality showed maximum in ICR males and C57BL/6 females than BALB/c mice of all bedding materials. Quantitative data analysis of ICR showed increased activity with maximum dirtiness of bedding attributed to higher body weights than other strains. Collectively, DVC data suggest that cage changing frequency may be increased up to 14 days without compromising animal welfare depending on its age, mice per cage and housing duration with different bedding materials in vivarium.

PC027

Cross-sectional Study in Argentina: First Approach to the Human-Laboratory Animal Interactions

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Abstract
The role of laboratory animals' care personnel implies caring for individuals that are almost completely dependent on them, with animals usually finishing their lives in the hands of their caregiver. This close and delicate interaction forges a bond between the human and the animals that frequently has a high personal cost. This devastating condition has been termed Compassion Fatigue (CF), a form of burnout typically affecting people in caregiving professions. Cross-sectional studies analyzing the development and impact of CF on the everyday work of professionals in charge of laboratory animals have been conducted in several countries. We aimed to investigate the prevalence of CF in Argentina and to identify risk factors in laboratory animal care professionals. We conducted a study between June and September 2024, following LaFollete et al (2020) survey. A total of 106 professionals completed an online questionnaire regarding social support, euthanasia, environmental enrichment, stress and pain inflicted, professional quality of life and human-animal interactions. This data will allow to determine how prevalent CF is in our country and to evaluate if factors like lack of social support, the possibility of deciding the method of euthanasia, or involvement in the selection of enrichment elements among others, could correlate with the development of this psychological syndrome. These results could provide empirical information to contribute to the development of intervention programs improving the quality of life of both the people who work with laboratory animals and the animals they care for, according to local conditions.

PC028

Evaluation of Simple Enrichment Elements on Mice Welfare by Behavioural, Neurogenic and Hormonal Factors

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Abstract
Environmental enrichment (EE) is the ensemble of elements in housing to facilitate natural motivated behaviors. Complex EE increases hippocampal neurogenesis and associated behaviors, probably influencing the outcomes of assays classically employed in neuroscience. Given that less is known about the effect of simple EE, we aimed to analyze its effect on male and female C57BL6/elite and SWISS mice. Animals were allocated into 4 groups: standard, complex EE (CE) and two simple EE (tube-T- or board house-BH-) housing conditions for 8 weeks. Observations in the home cage, anxiety and memory tests were performed. Neuron survival was evaluated in the hippocampus. Corticosterone was measured by enzyme immunoassay. Regarding the observations in cage in Swiss, CE and BH induced a significant decrease in stereotypical behavior ($p = 0.05$) in females. No significant changes were induced by the simple EE in the other behavioral tests, neither an effect on hippocampal neurogenesis. Concerning C57BL6/elite mice, preliminary results indicate that no main effect of simple EE was observed in either sex for anxiety or depressive-like tests. Additionally, a better performance in the memory test was induced in female mice only by the BH. Up to now, our results show that simple EE do not induce massive changes in behavioral tests commonly used in the field of neuroscience, neither in survival rates of hippocampal newborn neurons. However, the reduction in stereotyped behavior is indicative of improved animal welfare, suggesting that tube or board house could be added in mice cage to improve their welfare.

PC029

Routine Husbandry Management to Ensure Welfare and Health of Laboratory Dogs, Cats and Ferrets

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Abstract

To prevent boredom, fear, and stress in laboratory dogs, cats, and ferrets, environmental modifications and enrichment activities (e.g., outdoor access, human interactions) are recommended. These interventions aim to enhance coping mechanisms for everyday life events and facilitate potential rehoming. To address these aspects, current knowledge was reviewed on control and predictability from the animals' perspective, pre-arrival breeding conditions, on-site acclimatisation, socialisation, handling, human interactions, food and water management, housing, and environmental enrichment.

As suppliers of these species are limited, microbiological status is generally documented. Consequently, health management focuses on excluding clinically apparent and subclinical pathogens, alongside zoonotic diseases. While stringent hygienic measures control animal pathogens, emphasis on (anthropo-)zoonotic diseases is critical for staff safety. Proper training in hygienic procedures and adherence to standard operating procedures (SOPs) significantly reduce the risk of pathogen introduction and zoonotic infections. Staff must remain informed about zoonotic agents relevant to the species they handle, supported by annual training and routine SOP updates.

FELASA provides recommendations for health monitoring and reporting of these species. A FELASA member survey identified emerging pathogens that merit monitoring or consideration based on European seroprevalence, clinical risk to target species, impact on research reliability, and zoonotic potential.

Implementing these recommendations is expected to improve the welfare and health management of dogs, cats and ferrets, supporting both their well-being and research quality in routine laboratory care.

PC030

Automated Behavioral and Metabolic Phenotyping of Individual Rodents in a Social Group

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Abstract

Many promising preclinical studies in rodents fail in clinical trials, raising concerns about the efficacy of classical behavioral, physiological, and metabolic phenotyping approachs to generate novel treatment strategies. Several caveats and limitations play a critical role in this failure, most important: (i) rodents are nocturnal, but mostly tested only briefly and isolated during the light period; (ii) the isolated conventional experiments take only parts of a rich behavioral repertoire under group-housing conditions into consideration; and (iii) environmental factors, as well as experimenter influence are often underestimated. Undisturbed, 24/7 monitoring within environmental and/or social enriched conditions is obtaining increased attention and popularity to substitute and/or complement conventional phenotyping methods. Such strategies in accordance with the 3R (reduce, refine and replace) principles lead to increased animal welfare by reducing stress and data variability. Using different home-cage phenotyping systems, the IntelliCage and the PhenoMaster under group housing conditions lead to a better data reproducibility within and between laboratories. This approach will help to increase the translational value of preclinical behavioral and metabolic research.

PC031

When Can we Trust the Rat Grimace Scale?

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Abstract

The Rat Grimace Scale (RGS) is a widely utilized tool for assessing pain in preclinical research. However, it is often applied in isolation without validation against other behavioral pain assays. It is also often used past the less than 48 hours of pain onset – the period for which it was originally validated.

Although the RGS can easily be applied by trained personnel, it should be used cautiously when applied as a sole measure of pain. The foundational study on the RGS suggests that, even under ideal circumstances, 20% of assessments may be misleading. Such discrepancies highlight the need for caution when relying solely on the RGS. To improve the utility of the RGS, it is essential to compare its performance with other behavioral pain tests applied concurrently to the same rats. By systematically reviewing studies applying the RGS in combination with additional pain assays, we can analyze when these measures are no longer correlated. By applying novel meta-analytical methods, we have attempted to identify temporal limitations and potential inconsistencies in the RGS's performance. When can we rely on the RGS and when do we risk pain going undetected?

Any tool that can reduce suffering and contribute to the refinement of research is greatly welcome. However, a more robust application of the RGS enhances the reliability of preclinical studies while upholding the ethical imperative to reduce suffering.

PC032

In Vivo Imaging Monitoring of Corn Oil-induced Inflammation in C57BL/6J Mice

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Abstract
Corn oil is often used as solvent for the administration of lipophilic substances (e.g. tamoxifen) to animals. The oil solution can be applied either orally or by intraperitoneal injection. Our goal was to investigate the possible inflammation following 5-day administration of corn oil by using either intraperitoneal injection or oral gavage (metal ball-tipped or polypropylene plastic) by means of an in vivo imaging method in both male and female C57BL/6J mice.

Animals were intraperitoneally injected with the chemical substances luminol (100 mg/kg) and lucigenin (10 mg/kg) for the imaging of acute and chronic phases of inflammation, respectively. Luminol enables detection of acute inflammation mainly mediated by neutrophils, while lucigenin requires phagocyte NADPH oxidase activity in macrophages. Data and images were collected using the In Vivo Xtreme system (Bruker). Inflammation was evaluated based on the intensity of the emitted bioluminescence.

Intraperitoneal route of oil administration caused activation of the immune cells residing in the peritoneal cavity, resulting in intense acute and chronic inflammation, when compared to oral applications. Regarding the two types of esophageal needles, the metal ball-tipped induced mild acute esophageal inflammation in comparison with the polypropylene plastic ones. The results indicate that oral administration of corn oil is preferable due to minor inflammation induction and severity. In addition, in inflammation-immunological studies, possible impact of intraperitoneal corn oil administration should be always taken into consideration.

PC033

Experimental Zebrafish Husbandry of Safety Level 2 at the Maxplanck Institute for Infection Biology

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Abstract
Zebrafish (Danio rerio) are increasingly being used in biomedical research. In particular, the adaptive immune system, which is crucial for fighting tuberculosis in humans, first evolved in fish, and fish are therefore the lowest animals to possess the complete vertebrate immune system. Therefore, the zebrafish is also used as a model to study tuberculosis, the zebrafish M. marinum model. In this model, zebrafish are infected with Mycobacterium marinum, a closely related pathogen of M. tuberculosis (Mtb), which retains many of the virulence determinants present in Mtb and forms granulomas in the zebrafish that are very similar to those of Mtb in humans.

The use of M. marinum requires the fish to be kept in safety level 2. For this purpose, a special area has been set up in our institute that meets all the relevant requirements and is equipped with a ZEB TEC standalone active blue fish system from Tecniplast. An Enviro DTS system was installed to sterilize the waste water. Furthermore, certain hygiene regulation sand welfare criteria are adhered to.

PC034

Better Science by Tackling Antimicrobial Resistance with High-standard Asepsis in Experimental Rodent Surgery

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Abstract
Antimicrobial resistance is the ability of a bacterium to resist the action of an antibiotic. The excessive and often incorrect use of antibiotics has contributed significantly to the development and spread of resistant bacteria, leading some infections to be impossible to treat. In addition to this major public health issue, the use of antibiotics in research can lead to drug interactions with products tested on animals or disrupt their microbiome, confounding research. In experimental rodent surgery, avoiding the use of antibiotics therefore represents a true everyday challenge.

This presentation will develop our experimental performance-based approach to avoid the use of antibiotics by ensuring optimal asepsis during our operations. This challenging objective can be achieved by managing the four main sources of contamination:

- Environment, with proper separation of the various activities (care, anesthesia, surgery, and recovery workstations) associated with the forward-flow principle, strict disinfection of the various workstations and the use of sterile drapes.
- Instruments, by mastering the effectiveness of our sterilization methods (dry-heat, autoclave, glass-bead sterilizer), sterile cellophane use on large equipment and sterile consumables usage.
- Surgeons, by refined hand washing method, wearing sterile gloves, surgical masks, and a surgical gown.
- Animals, with a wide shaving combined with excellent skin disinfection. The use of chlorhexidine is more effective than other antiseptics in preventing surgical site infections.

This cessation of antibiotics use weave through the concept of 3R and Culture of Care and show our concern about the implication of our action on animal and human health.

PC035

Strain-Specific Behavioral Responses to Facility Maintenance Activities in Laboratory Mice

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Abstract
Introduction: Maintenance activities in animal facilities can introduce stressors like noise and vibrations. We investigated how simulated maintenance affects behavior in two mouse strains with differing stress sensitivities.
Materials & Methods: We exposed C57BL/6J (n = 6) and FVB (n = 5) mice to vibrations and 78 dB noise over 6 days. Locomotor activity was tracked using an infrared system before, during, and after interventions.
Results: Vibrations didn't significantly alter locomotion in either strain. C57BL/6J mice showed no notable changes during noise exposure. FVB mice exhibited altered activity trends during noise and significant dark-phase hyperactivity post-exposure ($p = 0.0137$), suggesting anxiety-related behavior. This hyperactivity persisted up to 48 hours, though not significantly. No sex-related differences in activity were observed.
Conclusions: Our findings reveal strain-specific responses to maintenance-related noise, with FVB mice displaying heightened sensitivity compared to C57BL/6J. This underscores the importance of considering strain variations when planning experiments during facility upkeep. Our study offers insights for minimizing research disruptions and maintaining animal welfare during necessary maintenance work.

PC036

Conflict Management in Laboratory Mice: Preliminary Results on the Role of a Synthetic Appeasing Pheromone

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Abstract
Conflicts and fighting, often escalating into aggression and violence, can be common in laboratory mice, posing challenges to animal welfare, experimental data reliability, and costs. This necessitates ongoing exploration of effective mitigation strategies.

To this end, an exploratory study was conducted on 100 RjOrl: SWISS male mice, divided into two groups: one continuously exposed to a synthetic appeasing pheromone (common trunk) and the other to a placebo. Behavioral assessments included the Elevated Plus Maze (EPM) and the Resident-Intruder Test (RIT), alongside physiological measurements of plasma corticosterone, serotonin, and testosterone levels. An assessment of clinical indicators of wellbeing was also performed.

Key findings revealed that plasma serotonin concentrations were significantly higher in treated mice. In the EPM, treated mice exhibited reduced vigilance behaviors, with fewer and shorter instances of "unsupported rearing". Similarly, in the RIT, "upright posture" behaviors, indicative of potential intimidation, were shorter in duration, although their frequency remained unchanged.

Additionally, the clinical assessment of wellbeing indicators revealed a notable trend, with the placebo group exhibiting a higher number of lesions related to fight wounds.

These preliminary results suggest that the synthetic pheromone can contribute to modulating aggressiveness and reactivity, likely through enhanced serotonergic activity, enabling more peaceful, effective social conflict management.

PC037

It's All about Big Data - How Digital Mouse Caging Advances the 3Rs

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Abstract
Undisturbed home cage recording of mouse activity and behavior has received increasing attention in recent years.

In parallel, several technologies have been developed in a bid to automate data collection and interpretation. Thanks to these expanding technologies, massive datasets can be recorded and saved in the long term, providing a wealth of information concerning animal wellbeing, clinical status, baseline activity, and subsequent deviations in case of experimental interventions. Such large datasets can also serve as a long-term reservoir of scientific data that can be reanalyzed and repurposed upon need. In this work, we present how the impact of Big Data deriving from home cage monitoring (HCM) data acquisition, particularly through Digital Ventilated Cages (DVCs), can support the application of the 3Rs by enhancing Refinement, Reduction, and even Replacement of research in animals.

PC038

Assessing Hygienic Working Conditions and Practices in Laboratory Rodent Surgery: An Online Survey Analysis

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Abstract
Surgery is an integral part of many experimental studies. Good surgical practice is a prerequisite for surgical success, optimal animal welfare, and it not only improves post-operative recovery, but also the overall outcome and validity of a study. Rodents – especially mice - are the most commonly used laboratory animals and the legal requirements to perform experimental surgery are identical for all species. However, minimum surgical training requirements vary significantly across countries, ranging from basic introductory courses in animal experimentation to supervised, advanced courses led by expert surgeons, which complicates efforts toward standardization. This study provides insight into surgical education and experience, available infrastructure, workplace satisfaction, and the application of good surgical practice in laboratory rodent surgery. Two online surveys with a total of 72 questions were distributed across Europe and 782 complete responses were received and subsequently analyzed. The results show that most researchers performing rodent surgery have no medical background. Furthermore, good surgical practice (i.e. sterile gowning and gloving, decontaminating and draping the patient and using sterile equipment) seems to be poorly implemented in rodent surgery. Additionally, half of all rodent surgeons have no assistance available, and most respondents expressed a desire for continued education and courses to deepen and refine their surgical skills. Consequently, training for rodent surgery should be tailored to the surgeon's pre-existing knowledge and additional surgical training should be made mandatory before performing surgery on laboratory rodents. This could improve both the animals' and the surgeons' welfare.

PC039

A Scoping Review on the Effects of Physical Enclosure and Accessories on Macaque Welfare

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Abstract
Altering the physical enclosure (e.g., size and complexity) and providing physical accessories (e.g., furniture, perches, or verandas) for species that respond to fear by moving upward, such as macaques, may benefit their welfare; but generalities are unknown. A systematic scoping review of literature on the physical environment's impact on macaque welfare included manuscripts from two databases. Articles were original research studies published in peer-reviewed journals evaluating the effects of physical environment on captive macaques. The search yielded 946 articles, screened by two researchers, with data extracted from the final 34 manuscripts by another researcher. Fifty-five percent of articles were published after 2000, and 65% focused on Rhesus macaques. In 60% of studies, monkeys were singly housed, 95% were conducted in a research setting, and 67% were housed indoors. Out of all the outcome measures, 92% were behavioral and 8% were physiological. No measures of affect were reported. Of the studies measuring abnormal behavior, 48% found positive outcomes from the physical enclosure or accessories while the rest showed no change. Less studied was proactive provision of enhanced physical environments to prevent abnormal behavior development. Four studies looked at the welfare impact of cage tier; 15/16 measures were not altered, while one found that monkeys on the bottom row expressed more stereotypies. Overall, there is a dearth of published research on the effects of physical enclosure and accessories. A comprehensive welfare assessment approach, incorporating physiology and affect measures, is needed to better tailor the physical environment to benefit macaque welfare.

PC040

Enhancing Rat Welfare: Implementing Low-Stress Handling Methods in a Large-scale Facility

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Abstract
Refining handling techniques is useful for improving welfare standards in rats and mice. Charles River Margate, a commercial breeder housing hundreds of thousands of rats and mice, faced the challenge of implementing low-stress handling methods across such large populations. Since 2019, Margate has actively promoted these techniques, successfully increasing their use among animal handlers without compromising efficiency or productivity.

While research suggests certain handling methods can induce anxiety in mice, the evidence with regard to rats is less conclusive. However, handling methods are still likely to have significant welfare implications in rats. Our study aimed to assess handling practices during cage changes in a population of over 100,000 rats of various ages and sexes, including both outbred stocks and inbred strains. Using a questionnaire, we identified the most common handling methods: shoulder saddle (40%) and cupping (26%) for adults, and chest and bottom handling (23%) for juveniles, with no differences between sexes.

Our findings indicate that the handling methods used at Charles River Margate are comparable to those used in non-commercial settings for adult rats. However, non-commercial facilities reported more frequent use of tail handling with juvenile rats. Further evaluation of handling techniques for laboratory rats would be useful for ensuring the best possible welfare outcomes for those animals.

PC041

Spontaneous Superficial Tumours in the NSG Mouse Strain

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Abstract
The NSG (NOD-*scid* IL2Rgamma^{null}) mouse is a severely immunocompromised mouse model with defects in the innate and adoptive immune systems, including deficiencies in T and B cells, functional NK cells and mature lymphocytes. The severe immunodeficiency allows the mice to be used in research in human immune function, infectious diseases, diabetes, oncology and stem cell biology. The mice are viable, fertile, normal in size and display grossly normal physical and behavioural characteristics. The NSG mouse is not susceptible to thymic lymphomas and can be used for short-and long-term experiments. NSG mice are good breeders with large litter sizes, and they can remain productive breeders for over a year.

In 2024, we monitored the occurrence of superficial tumours in our NSG mouse colony. Necropsies were carried out on over 30 mice carrying tumours, noting the age and sex of the mice and the location and size of the tumours. Tumours occur at an incidence of approximately 0.3% and represent 8% of all conditions reported for this strain. Over 90% of the tumours were found in female breeding mice and tumours were mostly located in the thoracic region; only a single tumour was present per animal. Histological investigation of some tumours determined them to be mammary tumours. The occurrence of these tumours has implications for the breeding life of the NSG mice, limiting it to 7 - 8 months of age.

PC042

What You Didn't Bargain for in Your Diet and Bedding!

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Abstract
Diet and bedding enter our facilities after being sterilised either by autoclaving or by irradiation to remove bacteria, viruses, protozoa, fungi and insect pests. Our diet is a fixed formula natural ingredient breeding diet and our bedding is spruce-based dried wood shavings. Unusual physical findings in diet and bedding are occasionally detected at unpacking or unsealing the diet or bedding bag. These findings typically lead to the diet or bedding being condemned as unfit for use and subsequently the affected bag is disposed of. Such findings are reported via our biosecurity system for investigation and resolution.

The unusual findings can be divided into animate and inanimate objects. One example of an animate object, mould, presents as a green to black discoloration and can occur as a single patch, but usually multiple patches are observed. Occasionally only a single bag is affected but sometimes it involves an entire pallet. The location of the mould is either on the packaging or directly on bedding/diet, or both. Such affected diet or bedding is disposed of. (Other examples of animate objects will be given.) Inanimate objects found in the diet or bedding, including a lollipop stick, silicone cone, nail and stone.

We will discuss the microbiological consequences of these findings and appropriate responses.

PC043

Training Good Surgical Practice for Rodent Surgery

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Abstract
Surgery is an integral part of many experimental studies. Good surgical practice (GSP) is a prerequisite for surgical success and optimal animal welfare. It not only improves post-operative recovery, but also the overall outcome and validity of a study. Rodents – especially mice - are the most used laboratory animals. Although legal requirements for performing experimental surgery are identical for all species, minimum training requirements to perform rodent surgery vary significantly. Therefore, we aimed to gain insight into the application of GSP during laboratory rodent surgery with surveys and a systematic review.

Most colleagues performing rodent surgery have no medical background and it must be assumed that they did not experience any surgical training during their education. Furthermore, GSP is poorly applied during rodent surgery and official guidelines are inconsistent and hardly available in Europe.

Consequently, dedicated training and guidelines must be developed and provided to improve the situation. Training for rodent surgery should be tailored to the surgeon's preexisting knowledge and additional surgical training should be made mandatory before performing surgery on laboratory rodents. This could improve both the animals' and the surgeons' welfare.

Our one-day course "Good Surgical Practice for Rodent Surgery"– combining lectures with practical hands-on parts – is a first step into this direction. Since 2022, more than 250 participants learned to perform surgical hand disinfection, sterile gowning and gloving, proper decontamination and draping of the patient and the correct usage of sterile equipment. The course is well received, and participants expressed their interest in more courses.

PC044

Extended Catheter Patency Using Cannulock Buttons in Rodents

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Abstract
The importance of the 3 R's in research—Reduction, Refinement, and Replacement—is an increasingly significant goal for the global

research community. According to the Foundation for Biomedical Research (FBR), 95% of all lab animals used are rodents, primarily mice and rats. Extending the use of research models and reusing them when appropriate is crucial for achieving the reduction and replacement of rodents in preclinical studies. Employing advanced technologies to develop animal models that enhance welfare is also a key consideration in planning and conducting research.

Repeated sampling and dosing in rodents, including long-term infusions, can be conducted more effectively using newly refined catheters and exteriorization methods. By using Cannulock buttons and medical-grade polyurethane catheters, we've extended catheter patency for long-term and crossover studies. The Cannulock button provides a closed system that easily attaches to any catheter, maintaining a reliable connection with its barbed stainless steel connector.

In our study, we used male Sprague-Dawley rats. The Cannulock buttons were accessed twice weekly for 90 days post-surgery. Twice a week, we removed the locking solution and flushed the catheters. After flushing, we withdrew a small amount of blood to perform a full patency check. At the end of the 90 days, all animals were euthanized, and a necropsy was conducted. The results confirmed that we were able to extend patency to 90 days. We also observed improved welfare for both animals and technicians due to the ease and less stressful method of accessing catheters.

PC045

Long Term Rodent Catheter Patency – Myth or Reality?

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Abstract
Once surgery is complete and the catheters are implanted this starts your journey down the catheter maintenance and patency road. Completing a good surgery is just the beginning of a successful study and one largely underestimated portion of this research model is protecting and properly accessing the catheter or implanted device post-surgery. During this presentation we wanted to provide some essential procedures to aid in keeping catheters patent for longer periods of time. We will discuss recommended situations that using a catheterized model would be beneficial, various catheter materials and their uses, different methods for exteriorizing the catheters, recommended flushing and locking solutions as well as frequency of flushing and troubleshooting a problematic catheter. The presentation will conclude with some tip and tricks to help make using a catheterized rodent model easier.

PC046

Still a Place for Microscopy: Comparison of Techniques for Diagnosing *Myocoptes musculinus* in Mice

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Abstract
The detection of murine fur mites remains challenging, not least because they commonly cause no clinical signs in infected mice. A comparison of commonly used methods was performed on 29 mice with a mixed genetic background aged between 11 and 30 weeks. Methods tested were: fur PCR, tape strips (ventral and dorsal pelt, using standard and extra-strength tape), hair pluck (inguinal, ventral neck and behind ear), inguinal skin scrape, and tape applied just after death and left in place for at least 5 hours. PCR on faeces from infected cages was also tested.

All animals in the study were positive by at least one method. Mite burden did not show any clear correlation with age, but was similar between cage mates. Contrary to other studies, PCR testing was not the most sensitive method for mite detection (fur PCR 69%). Only tape applied after death for 5 hours had 100% sensitivity, closely followed by inguinal hair pluck or skin scrape (both 97%) and tape strips (90–97%).

Faecal PCR sampled from the cage closely matched the fur PCR results from individual animals and represents a good screening alternative to fur swabs that eliminates handling stress.

PC047

3Rs in Action: Insights from Standardising Health Screening at a Large UK Academic Institution

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Abstract
Health screening plays a crucial role in managing laboratory rodent colonies to prevent ill health and subclinical infections that could impact research results. The University of Oxford has been using dirty bedding live sentinels to screen for infectious agents, using approximately 1550 mice and 20 rats annually. We trialled the feasibility of moving towards non-sacrificial screening by PCR, testing dirty bedding shake cages, AHU filters and plenum swabs. We found that dirty bedding shake cages provided the most consistent results and that these were comparable with the live sentinel results Dirty bedding shake cages also proved more sensitive for *Rodentibacter* and *Myocoptes* than dirty bedding sentinels.

As a result of the trials, the University has committed to ending the use of live sentinels in early 2025, moving completely to non-sacrificial screening. Simultaneously we are moving all nine rodent facilities to a streamlined screening regime to facilitate animal movement between facilities within the University. This process has required clear and timely communication of all changes to veterinarians, animal care staff and researchers.

PC048

Reimagining the Role of the Colony Technician

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Abstract
The University of Dundee has implemented a new genotyping and colony management system to improve efficiency and accuracy.

Previously, genotyping was typically performed on ear notches 'in-house' within research groups by junior researchers, using PCR. Results were then entered into a colony management system, and researchers decided breeding and culling strategies based on the genotypes.

To streamline this process, the University partnered with an external genotyping service. Animal technicians now collect ear tissue samples and place them into 96-well bar-coded plates. Trained biological services staff (BSS) enter animal and line identification information into an online submission system. Plates are sent to the external service, where genotyping is performed using a highly accurate and precise robotic system. Results are uploaded to the online system, checked for inconsistencies by BSS and then made available to researchers and BSS colony managers (senior animal technicians who oversee breeding). Working with researchers, based on written colony management agreements, colony managers allocate animals for breeding or other purposes as required.

This new system has been widely adopted and handles >900 samples each month, from approximately 100 lines. It has improved efficiency in all aspects of the process. The turnaround time for results is typically less than five days, allowing prompt culling of unwanted animals and early grouping of experimental animals, improving welfare.

Overall, the system has significantly improved accuracy and efficiency of genotype identification, enhanced communication between researchers, NACWOs, DV and facility staff and allowed technicians to use their expertise, leading to better job satisfaction.

PC049

Behavioral Analysis: A Tool to Improve Care and Welfare in a Ferret Facility

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Abstract
Istituto Zooprofilattico Sperimentale delle Venezie is one of the few Institutions in Europe and Italy using ferrets as animal model to study influenza and other respiratory viruses. Due to the limited number of authorized suppliers and problems related to their transport, our researchers usually purchase large batches of animals. Therefore, in our facility male ferrets live in two large social groups (n = 8 and 12), differing in age and provenience (*i.e.*, supplier). Each group lives in a large, enriched enclosure (10.5 m^2), in which ferrets have access to a refuge/den area or can explore the environment, provided with tunnels, balls and other toys. Besides, a caretaker enters daily the enclosure for cleaning operations. Thanks to such non-stressful interaction with caretakers, ferrets do not fear handling and are thus less stressed during experimental procedures.

To evaluate the ferret welfare under present conditions, we developed a detailed ethogram comprehending a variety of ferret behaviors, such as eating/drinking, walking, running, self- and allo-grooming, sleeping, solitary and social play, agonistic behaviors, vocalizations and eventual stereotypies. We used a one-zero sampling to analyze the one-hour videos recorded after the completion of daily cleaning operations. By means of a detailed behavioral analysis, we described the behavioral profiles of the two ferret groups. To our knowledge, this is a first attempt to monitor ferret behavior when in large groups, since they are commonly pair-housed. As adequate space and sociality are crucial when considering animal welfare, our findings can be useful in programming effective interventions for appropriate ferret housing.

PC050

Establishment of Naked Mole Rat Facility at the CECAD

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Abstract
Introduction: Currently, there is no standardized method for keeping naked mole rats. To ensure their welfare, essential information was gathered and evaluated to meet their specific needs, focusing on optimal conditions such as constant temperature, humidity, and a natural underground habitat with tunnels.
Materials and Methods: The project had two main goals:

- Developing a modular tunnel system.
- Constructing an enclosure that maintains stable temperature and humidity.
- Recycled polycarbonate cages (Thoren) were modified with 3D-printed components. The cages were fitted with openings and sliders for flexible connections with tubes to prevent escape. This modular system allows for easy adjustments in housing design. To control the climate, lids were fitted with air holes to reduce heat loss while maintaining air circulation. A five-week test ("Empty Run") monitored humidity and temperature stability.
- Two enclosure designs were tested:
- A prototype that aimed to regulate all parameters but struggled with humidity and heat control.
- A customized frame with insulated walls, doors, and integrated heating mats that successfully maintained the desired conditions.

Conclusion: Creating a suitable naked mole rat enclosure requires careful planning and testing to avoid negative impacts on breeding and care. Keeping humidity levels consistent is particularly challenging, with weather fluctuations affecting this. In colder months, additional humidifiers may be needed. The modular tunnel system offers flexibility in adapting colonies, making management easier. This project provides valuable insights for standardizing care conditions and improving animal welfare in naked mole rat research.

PC051

Ammonia Concentrations in Different Sizes IVC Cages

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Abstract
Smaller and smaller cages are being used to house mice for economic and management reasons. But what does this do to the air quality and microenvironment when cages become smaller? Cage size and housing density in Individually Ventilated Cages (IVC) are, together with ventilation rate, determining air quality of the microenvironment inside the cage. Together with temperature, humidity and carbon dioxide, ammonia is an important factor for the microenvironment. Increasing ammonia concentrations in small cages may form a risk for the animal's health and wellbeing, and can cause various pathologies in the animals from chronic exposure. To map the risk of ammonia build-up in the smallest available IVC cage at our facility (Emerald line, Tecniplast) with different housing densities and a 14-days cage change cycle, we measured ammonia concentrations with a photoionozation detection sensor and compared them to other IVC cages. We found that most cages, 68–100%, exceed 20 ppm, and 21–42% of the cages exceed 50 ppm.

PC052

The Use of RADAR to Detect Vital Signs in Laboratory Animals

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Abstract
Most methods for monitoring vital signs in animals require contact in some form, which may not be accepted by the animal. In certain instances, this may be resolved with training, anaesthesia or handling but these are all likely to introduce artifacts. Even in human subjects, measurement of blood pressure or pulse can induce states which lead to exaggerated responses and misdiagnosis (e.g. "white coat hypertension"). In laboratory studies, there are many instances where regular monitoring of vital signs would be beneficial for the understanding of temporal or diurnal aspects of drug effects. Ideal would be systems that could record animal state many times daily without interfering with their freedom to move or interact (i.e. maintaining a social environment). We are evaluating various forms of RADAR as a means to detect vital signs without the need to contact animals or place telemetry instruments or sensors. High frequency radar is reflected by skin surfaces. Vibration or movement in the skin surface due to breathing or pulse is detected as a variation in the distance between the emitter and the reflector. Due to the wavelengths being in the µm range, even subtle movements in skin surface are detectable. Traces, thus detected, can be used to estimate animal state without the presence of a human observer. Our group is in the process of refining this technology to allow it to make more useful observations of laboratory animals. The non-contact aspects suggest significant potential benefits for monitoring animals.

PC053

Modest Differences in Mental and Physical Health of Male Mice under Different Housing Conditions

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Abstract
Mice are social animals and typically live in groups, but in the laboratory environment that is not always possible due to experimental requirements or to aggressive behavior. At ambient temperature, individually housed mice experience welfare issues as, cold stress and social interaction. We provided individually housed male mice (C57BL/6J) with a heating plate, to compensate for the lack of warmth from a cage mate or used a transparent semipermeable wall dividing a cage in two compartments to allow olfactory, visual, and acoustic communication between two mice. Long-term effects on physical and stress related health were investigated and compared to an individually and socially housed group.

Preliminary results suggest that after 12 weeks in the different housing conditions socially housed mice, compared to individually housed mice, showed less fat per gram bodyweight, ate more and showed an increased femur size. This suggests that socially housed mice display a better physical health. Socially housed mice had higher corticosterone levels and entered open arms more frequently on the elevated plus maze. Although increased corticosteroids suggest an increased stress level (~1.5 fold), they were less anxious on the elevated plus maze compared to individually housed mice. Interestingly, providing a heating plate or access to social interaction (via the transparent semipermeable wall) did not impact these physical and mental health parameters in individually housed mice.

Our results suggest that the adapted housing conditions didn't improve health of individually housed mice. Further investigation is needed to optimize housing conditions for individually housed laboratory mice.

PC054

Use of Vascular Access Buttons for Drug Administration and Blood Sampling in the Rabbit

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Abstract
Researchers are increasingly turning to Vascular Access Buttons (VABs) as their go-to method for refining blood sampling and drug administration. Unlike traditional methods that involve repetitive venipuncture, which can be distressing for rabbits and lead to bruised and sensitive ears, VABs provide a more humane approach. While chronic vascular access models in rabbits often involve exteriorized vascular catheters and implanted access ports, the use of VABs in rabbits has been less explored.

In a recent study, rabbits were implanted with a single channel VAB, with the catheters being inserted into the jugular vein. The animals showed excellent tolerance towards the VABs, with minimal reported issues and little resistance during catheter maintenance, dosing, and sampling. The use of VABs offers significant benefits to researchers by eliminating the need for repeated needle punctures, providing a less distressing method for rabbits to receive long-term treatment. The use of VABs has a positive impact on animal welfare as it reduces the severity of previous methods of venipuncture and reduces the risks of infection and other associated complications.

Our team advocates for the use of Vascular Access buttons (VABs) as a superior alternative to traditional blood collection methods in rabbits for PK/PD studies due to their improved ease of use, reduced stress on the animals, and increased study efficiency. While exteriorized catheters offer an alternative, their associated risks and limitations make VABs a more favourable option.

PC055

Refining Surgical Training: An Easy-to-Set Up Fabric Mouse Model

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Abstract
Introduction: The 3Rs are increasingly being put into practice in laboratory animal research. However, we are still a long way from eliminating the use of laboratory animals, so refinement is essential. To this end, simulation-based learning helps trainees to acquire proper techniques before working with animals. However, appropriate models might be expensive or difficult to find. Here, we propose an easy-to-set up fabric mouse for surgical training.
Objectives: - Develop psychomotor skills, visual acuity and dexterity
- Reduce stress by creating a fun environment
- Train learners to objectively evaluate a peer
- Provide an easy-to-set up and inexpensive workshop

Methodology: We set up a model of a mouse abdomen with fabric and stuffing, containing various tiny objects of daily life. One student in each pair must take it in turns to use the surgical instruments correctly to open the fabric mouse, take out the objects and sew it up. During this operation, the other student assesses his/her partner according to a grid of criteria.

Key points: This fabric mouse was used with several groups of students and has been found useful to improve the acquisition of technical skills in surgery, in a positive atmosphere.
Conclusion: We present here an easy-to-set up, adaptable and inexpensive workshop to improve the skills in surgical training, for an improvement of the welfare of laboratory animals and the staff working with them.

PC056

Burkholderia gladioli: Presentations and Diagnosis of an Emerging Opportunistic Pathogen of Immunocompromised Rodents

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Abstract
Severely immunocompromised rodents are susceptible to opportunistic bacterial infections. Recent literature and diagnostic case experience have shown that one such opportunist, *Burkholderia gladioli*, can result in various clinical presentations which are devastating to research colonies. While primarily known as soil-associated plant pathogens, B. gladioli and other non-melioidal *Burkholderia* species have also been implicated in human infections, including those complicating cystic fibrosis cases, and causing respiratory infections or bacteremia in immunocompromised patients. In rodent colonies, rapid identification of causes leading to declining health in immunocompromised animals helps speed the investigative process to determine the potential breach of biosecurity and infection origin. In most cases of opportunistic *Burkholderia* spp., rapid identification has allowed intervention to eliminate the source of infection with a return to research. This talk will review common clinical presentations, diagnostic evaluation, and potential elimination strategies for *Burkholderia* gladioli and similar opportunistic bacterial infections in immunocompromised rodents.

PC057

Current Microbiological Status of Mice and Rats in Experimental Facilities in Japan

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Abstract
Our center is a third-party diagnostic laboratory for microbiologic and genetic monitoring of laboratory animals, and has been performing tests over 45 years in Japan. In 2024, a total of 25,379 mouse samples and 2,627 rat samples were tested from 2,359 mouse facilities (2,137 universities and research institute: U/I,

222 Pharmaceutical companies and CROs: P/C) and 454 rat facilities (376 U/I and 78 P/C) in Japan. Test results were compiled by facility (number of positive facilities/number of testing facilities) for each item. In mouse facilities, the following bacterial and fungal items were positive; *Staphylococcus aures* (Sa: 15.1%), *Pasteurella pneumotropica* (Pp: includes two species of *Rodentibacter* and related strains, 3.2%), *Helicobacter* spp. (2.7%), *Pseudomonas aeruginosa* (Pa: 2.3%), *Helicobacter hepaticus* (0.1%) and *Pneumocystis murina* (0.1%). In viral items, only MHV was positive (0.2%). In the parasite items, multiple intestinal protozoa (0.1%-5.2%) and pinworms were positive (*Syphacia* spp.: 1.1% and *Aspiculuris* spp.: 1.2%). In rat facilities, the following bacterial and fungal items were positive; Sa (40.0%), Pa (7.7%), Pp (1.6%) and *Mycoplasma pulmonis* (Mp: 0.2%). In viral items, no positive results were found. In the parasite items, multiple intestinal protozoa (0.2%-4.2%) and *Syphacia* spp. were positive (1.8%) in pinworms.

These results indicate that no outbreaks of serious pathogens that could be fatal to mice and rats have been observed in mouse and rat facilities in Japan, and that common positive cases were due to opportunistic pathogens and intestinal protozoa.

PC058

A Sentinel-Free Soiled Bedding Sampling Alternative to Direct Animal Sampling for PCR-based Rodent Quarantines

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Abstract
A substantial risk that can introduce pathogens into a rodent colony is the import of rodents from outside sources. Direct colony PCR sampling (DCS; feces/oral swab/pelt swab) of quarantined rodents is commonly used for infectious agent detection. Sentinel-free soiled bedding (SFSB) sampling by exposure to a contact media for PCR testing has been adopted by a growing number of institutions as a routine method for colony screening. We investigated SFSB as an alternative to DCS for quarantine because it could eliminate undue animal stress during sample collection and eliminate compassion fatigue and labor time required by animal staff to collect DCS. An initial pilot study with conventionally reared mice demonstrated equivalent or superior detection of agents by SFSB versus DCS. A subsequent main study evaluated 30 imported quarantine mouse strains from outside institutions over an 8-months period by both SFSB and DCS. An accumulative 19 different genus or species PCR infectious agent assays were positive among both methods with 157 and 173 total assay positives for DCS and SFSB, respectively. Although DCS and SFSB were equivalent for most agents, SFSB had statistically improved detection of *Klebsiella pneumoniae*, *Proteus mirabilis* which were undetected by DCS. By contrast, *Bordetella pseudohinzii* was only detected by DCS. Hands-on collection of only one sample type and reduced animal manipulation simplified the SFSB process compared to DCS. SFSB is a refined alternative to DCS for detecting rodent pathogens in quarantine that eliminates unnecessary animal handling during sample collection, which reduces stress for rodents and animal staff.

PC059

Exploring Histopathological Phenotypes in Aging Nothobranchius furzeri: Insights from an Integrated Health Management System

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Abstract
The short-lived African killifish *Nothobranchius furzeri* offers unique advantages for aging research, yet little is known about its age-related histopathological changes. Understanding these phenotypes is crucial for advancing animal welfare and improving scientific outcomes. This study systematically characterizes key age-related histopathological features, including tissue degeneration, neoplasias, and inflammatory responses, to establish a comprehensive pathology framework for this species (1).

To enhance health monitoring and welfare management, we integrate these insights with *Fishlifelines*, a custom software tool linked to the tick@lab system, which visualizes survival and reproductive success. This allows dynamic health assessments and early identification of emerging health issues in colony management. Correlating pathological changes with reproductive performance and lifespan supports refinements in breeding strategies, husbandry protocols, and enrichment practices aimed at reducing stress and enhancing resilience.

By combining detailed histopathological analysis with data-driven health management, we address knowledge gaps in aging research while promoting proactive welfare measures. This approach underscores the importance of continuous health surveillance and pathology-based refinement strategies to optimize both animal welfare and research quality.

PC060

Optimized Breeding Strategies for *Nothobranchius furzeri*: Enhancing Welfare and Reproducibility

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Abstract
Nothobranchius furzeri, also known as African Turquoise Killifish is a widely used model in ageing research. Breeding of this short lived vertebrate presents specific challenges that impact both animal welfare and experimental reliability. This poster focuses

on key refinements in breeding protocols to optimize spawning success, preserving genetic diversity and enhance welfare. Our welfare-centered breeding strategy employs harem-based mating with up to four females per male, significantly reducing stress of females compared to one-to-one pairings. Continuous mating further promotes natural reproductive behavior without imposing rigid schedules.

Critical challenges are maintaining genetic diversity and avoiding inbreeding depression, as wild populations of *N. furzeri* are not accessible for genetic refreshment. We address this limitation by carefully managing breeding and minimizing genetic drift through balanced breeding group selection. To increase reproductive efficiency and resilience, we implement several parallel breeding groups of different ages and from different parents. Based on our health score sheet (1), our breeding protocol prioritizes the selection of healthy, robust breeders to maximize embryo viability. We identified an optimal age range for breeders that correlates with improved embryo quality and hatchling survival. By integrating enriched group spawning setups, we further promote welfare and natural behaviors.

By integrating welfare-driven mating strategies, and genetic management practices, we achieve higher reproductive success, reduced variability, and sustainable colony management. This approach aligns with the 3Rs, improving both animal welfare and scientific outcomes. Our work serves as a model for ethical breeding and care in research involving short-lived fish species.

PC061

Setting Up Your Surgical Field for Surgery in Pigs and Stereotaxic Surgery in Rodents

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Abstract
The goal of aseptic technique is to reduce or eliminate where possible the normal bacterial burden present on the animal and in the environment before beginning surgery. This means that instruments must be sterilized, the surgical working surface is disinfected, and the animal and the surgeon's hands are disinfected. Proper aseptic technique limits the risk of introduction of infectious agents, primarily bacteria, into surgical sites.

Setting up your surgical field before surgery is crucial for a successful aseptic surgery, even in experimental surgery. It is absolutely essential to practice surgery in aseptic conditions as it would he done in veterinary surgery or in human surgery. The reasons are of course the same for the patients themselves, but also in order to limit biases in results obtained, which is the absolute aim of research.

Here we will present a step guide to prepare your surgical field for both large and small mammals, with two different kinds of species, e.g. pig and rodents.

PC062

Myth Busting: The Truth About *Corynebacterium* Bovis

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Abstract
Corynebacterium bovis, a bacterium commonly associated with bovine intestinal flora, has become the subject of various misconceptions regarding its presence and impact in laboratory rodents. This presentation aims to debunk common myths and provide an evidence-based perspective on the bacterium's relevance in immunocompetent laboratory rodents. Firstly, it is believed that C. bovis is a prevalent contaminant in rodent vivaria, posing a risk of zoonotic transmission and compromising experimental validity. A comprehensive review of microbiological data from controlled vivarium environments reveals that C. bovis is not a typical resident of laboratory rodent microbiota. Additionally, experimental analyses confirm that C. bovis has a limited capacity to colonize immunocompetent rodent hosts, contradicting the notion of its ubiquity and pathogenic potential in these models.

Another myth suggests that the presence of C. bovis in rodent studies may significantly alter the outcomes of gastrointestinal or metabolic research. However, several studies indicated minimal interference with physiological processes in rodents, even in experimental inoculations designed to mimic high exposure scenarios. This suggests that concerns regarding its impact on study's reproducibility and validity are largely overstated.

This presentation also addresses misconceptions about detection methods, highlighting that modern molecular techniques are robust enough to differentiate between C. bovis and other Corynebacterium species, mitigating the risk of misidentification. In conclusion, our review demystifies several myths surrounding C. bovis in laboratory rodents, emphasizing the need for accurate microbial characterization and caution against overestimating its role in experimental settings. This clarity is crucial for maintaining the integrity of rodent-based research.

PC063

Can Tail-handling be Non-Aversive? A Comparative Assessment of Mouse Handling Techniques

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Abstract
This study aims to investigate whether tunnel handling provides superior welfare outcomes compared to tail handling in mice. In the mouse breeding core of Novartis Pharma AG in Basel, Switzerland, an alternative handling technique, referred to as "refined tail-handling," has been implemented. The calm behavior exhibited by the mice even during routine handling has prompted a

hypothesis that refined tail-handling is not inferior to tunnel handling in terms of animal well-being.

To test this hypothesis, we conducted a comparative analysis of breeding performance between mice subjected to tunnel handling and those handled with the refined tail-handling technique. Breeding performance was chosen as a surrogate marker of animal well-being due to its established correlation with the overall health and stress levels in laboratory rodents.

Our findings demonstrate that despite the routine tail handling utilized in our facility, the breeding performance of mice remained largely unaffected, suggesting minimal aversive effects associated with the refined tail-handling technique. These results support the notion that refined tail-handling could be a viable alternative to tunnel handling, providing a more practical approach that does not significantly interfere with established animal care procedures, such as regular health checks.

This study contributes to the ongoing efforts aimed at refining animal handling methods and prioritizing the welfare considerations of laboratory mice while maintaining operational efficiency. Further research is warranted to explore additional indicators of animal well-being and validate our findings across different mouse strains and research settings.

PC064

Permanent Small Intestinal Cannulation in Göttingen Minipigs – Technique and Management

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Abstract
The direct delivery of compounds to the small intestine can be a great help in pharmacological research, avoiding the influence of stomach digestion involved in delivering orally and the use of anesthetics, involved in delivering by endoscopy. This study presents a technique to permanent small intestinal cannulation in pigs, designed to deliver large-sized tablets, pillcams and endoscopes directly into the intestinal lumen, and it also facilitates intestinal content sampling.

We have to date operated 24 female pigs weighing between 19,3 kg and 28,7 kg with an average of 22,4 kg. To ensure the success of postoperative management, a comprehensive presurgical socialization and training regimen was established for the animals. This included the implantation of a permanent vascular access catheter for stress-free blood sampling, a tailored feeding schedule before surgery, and a postoperative feeding and analgesia regimen to prevent complications such as constipation and volvulus.

Out of 24 cannulated animals, 19 procedures were successful with no complications related directly to the cannula function. On average, the animals were utilized for experimental purposes for a duration of 5.8 months, reaching up to 9.7 months. The two main reasons for terminating the animal were that the animals had participated in enough projects and outgrowing of the cannula by the animals as the abdominal wall increased in thickness.

This was only possible due to the comprehensive animal management protocol . The poster will detail the innovative cannula design, surgical techniques, perioperative animal training and care procedures that contributed to the success of this study.

PC065

How to Act When Detecting Tumor Ulceration in Mice within Cancer Research

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Abstract
Ulceration of subcutaneous tumors in mice within cancer research with subcutaneous tumors is seen on a regular basis within animal facilities.

For animal welfare reasons, quality of research and to make sure the severity of certain procedures remains within the allowed severity level of the permit, a procedure was implemented how to act when dealing with tumors that may ulcerate.

Timely detection of early stages of tumor ulceration, short lines of communication between the staff of the animal facility - research groups - Animal Welfare Body and rapid decision-making are important for this.

This poster describes the policy and refinements implemented in the occurrence of ulceration of subcutaneous tumors in mice within animal experimental research.

PC066

Enhancing Inhalation Study Outcomes Through Mouse Training Programs at AstraZeneca

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Abstract
AstraZeneca has developed an inhalation training program for mice, enhancing both animal welfare and the reliability of inhalation studies. This program prioritizes gentle handling and positive reinforcement, acclimating mice to the inhalation process to reduce stress and improve data accuracy. Initially, mice familiarize themselves with the inhalation tube in their home cage, supported by a rotating enrichment schedule and play table to stimulate curiosity and prevent neophobia. Gradually, mice are introduced to the lab environment, acclimating to its sounds and smells, which significantly reduces stress behaviors during dosing.

Our refined training method aligns with the ethical 3R framework (Replacement, Reduction, and Refinement), involving one to two weeks of short training sessions where animals calmly adapt to lab procedures, including restraint. Positive reinforcement ensures normal breathing, crucial for reliable plethysmography

data. This approach uses a new inhalation system that requires 15 times less test material, facilitating sensitive respiratory parameter measurements.

By ensuring mice are well-trained and acclimated, we can detect subtle respiratory changes early in drug discovery, reducing animal numbers while maintaining data integrity. This strategy enhances robust plethysmography assessments across our inhaled drug portfolio, de-risking and prioritizing compounds efficiently. AstraZeneca is committed to ethical practices and efficient drug development, ensuring more consistent and reliable outcomes in inhalation studies.

PC067

Emotional Baggage: What Can We Do about It?

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Abstract
Working directly with animal in science can have an impact on the operator's mental health. From compassion fatigue to care killing paradox, many of us carry a professional emotional baggage of some sort.

This presentation will give a quick overview of the challenges then offer some tools to cope with them, both from a personal and an institutional approach.

PC068

Refinements for Cranial Implant Application in Macaca Fascicularis

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Abstract
Cranial implants are essential tools in neurophysiological studies involving non-human primates (NHPs). However, their application requires major surgery under prolonged anesthesia, with a notable risk of inaccurate positioning of the implant.

This study aims to describe advanced techniques to improve cranial implant procedures for two macaques (*Macaca fascicularis*) involved in neurophysiological research in the Laboratory of Neurophysiology of the Visuomotor System at University of Bologna. Preoperative Magnetic Resonance Imaging (MRI) and computed tomography (CT) were conducted under intravenous anesthesia to identify surgical landmarks and guide the fixation of implants. Using the CT data, 3D-printed models of the skulls were created for procedural planning, allowing for precise preparation of surgical instruments and materials before in vivo application. During surgeries, animals were kept under intravenous anesthesia combined with local-anesthetic blocks of the scalp, and vital parameters were continuously monitored. Intraoperatively, a markerless neuronavigation system (CORTEXPLORE SCI; cortEXplore GmbH, Linz, Austria) provided real-time visualization of the virtual models of the brain, the skull, and the implants to be placed, ensuring accurate positioning of the implants.

The described techniques allowed the preparation of customized implants tailored to each subject's anatomy, improving surgical efficiency and achieving precise targeting of areas of interest with an error <0.3 mm. These advancements represent a significant refinement in neurosurgical techniques for NHP research, promoting animal welfare while enhancing the precision and reliability of scientific outcomes.

PC069

Local Anesthetic Techniques for Thoracic Surgery in Pigs

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Abstract
Pigs are common models for testing medical devices requiring thoracic surgery with multimodal analgesia . We described three different local-anesthetic (LA) techniques applicable for these procedures, considering their feasibility, advantages and disadvantages. Twenty commercial hybrid pigs undergoing sternotomy for testing an extracorporeal circulation device were considered. In the isoflurane anaesthetized animals receiving fentanyl infusion, the following LA techniques were applied: none, blinded bilateral intercostal (IC) blocks and lumbar (L4-L5) spinal (SA) or epidural anesthesia (EA) anesthesia. Analgesia was considered inadequate if cardiovascular variables increased by 20% above the preoperative values.

The three LA techniques resulted in superior analgesia as compared with absence of LA techniques. The IC blocks were the easiest to perform, however, drawbacks consist in the larger volume of drug administered and in the longer time interval necessary to complete the procedure if compared with the other two techniques. The correct needle placement for the blinded IC blocks relied only on the anatomical landmarks. Both the SA and the EA required more training, but they were faster once the skill was acquired. In these cases, the correct needle placement was confirmed based on the anatomical landmarks and by the appearance of the cerebrospinal fluid or with the hanging drop technique respectively for the SA and the EA.

The LA techniques represent a refinement for analgesic management of pigs undergoing sternotomy; however, the identification of the anatomical landmarks cannot be overlooked, therefore their feasibility is limited by the body condition of the animals, being more difficult in obese pigs.

PC070

Genetic Assays as Tool for Conservation Management of Non-human Primate Species

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Abstract
The breadth of genetic diversity in immune response genes within a population is of great importance to minimize the possibility that a population is wiped out by one pathogen. In addition, knowledge on the parentage and geographical origins of a species may enhance colony management and opens ways to control exchanges between different populations, for instance to prevent inbreeding.

In the past two decades we have developed several assays to characterize the genetic diversity in great ape, Old and New World monkey, and lemur species. One of the assays enables a quick and robust characterization of the major histocompatibility complex (MHC) DRB gene profile. The MHC region is highly polymorphic and polygenic, and encodes molecules that play a crucial role in immune responses. Our platform has proven to be widely applicable to multiple species, and can be used to monitor and manage colony or population composition. Next to invasive techniques, such as drawing blood, this protocol can also be applied using DNA isolated from non-invasive sources, such as hair and feces. Furthermore, validated assays are available for origin determination by mitochondrial DNA analysis as well as for parentage definition by selecting for up to 23 different polymorphic microsatellites.

In conclusion, these assays offer valuable tools for enhancing the conservation management of non-human primates, supporting their long-term survival and well-being, and enabling to assess and preserve genetic diversity within populations.

PC071

Ways to Extend Catheter Patency Duration and Improve Animal Welfare in Rodents

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Abstract
Access to the vascular system is a critical aspect of many research studies. For infusion of compounds, blood withdrawal or both, an ample period of catheter patency is necessary for chronic studies. Multiple factors affect catheter patency duration, the most important being catheter tip placement. The catheter tip must be positioned in the ideal location for optimal patency. Having a closed system is the second factor. Studies have demonstrated that a closed system using transcutaneous buttons has a major impact in extending patency duration. Catheter material and tip profile also affect patency. Polyurethane is the optimal material and a round catheter tip minimizes damage to the endothelial cells of the blood vessels, thus reducing the formation of blood clots at the catheter tip. Adhering to a strict aseptic technique and using sterile solutions will negate biofilm formation at the catheter tip supporting an extended patency. Another way to support patency is the positive pressure technique when locking the catheter. Utilizing this technique reduces the probability of clot formation within the catheter. The transcutaneous button not only supports an extended patency duration but also improves animal welfare. With the addition of the metal cap on its port, group housing is possible, allowing rodents to express their social behavior. Additionally, the button reduces animal handling and associated stress, subsequently promoting better research results. Furthermore, by increasing catheter patency duration, we can reduce the number of animals undergoing a surgical procedure as a high enrollment is no longer necessary for chronic studies.

PC072

Detection of Pre-weaning Health in Mice Using Home Cage Activity Monitoring

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Abstract
The use of non-invasive home cage monitoring provides continuous information about behaviour and health in mice, that cannot necessarily be detected during daily welfare checks. By using the digitally ventilated cage (DVC, Tecniplast), a commercially available monitoring system, we measured locomotor activity levels within the home cage during routine breeding in C57BL/6J mice. Our emerging data indicates that we are able to detect healthy and unhealthy litters pre-weaning; providing a marker for the health of the pups as early as P9. Cages with healthy litters show activity throughout both light and dark periods from P9, with consistent activity until weaning age. By contrast, this characteristic pattern of activity emerges much later in unhealthy litters, with lower overall activity until weaning age. These findings suggest that home cage monitoring systems can effectively capture subtle differences in locomotor activity, which can be an early indicator of health status in laboratory mice. The use of DVC has the potential to provide earlier detection of disease progression, and behavioural phenotyping through subtle differences in activity levels across the day/night cycle, as well as potential to refine breeding strategies to improve the health and welfare of laboratory mice.

PC073

Management of Newborn Triplets in a Marmoset Breeding Unit: Example of a Successful Approach

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Abstract
In captivity, marmosets very often give birth to triplets or more, unlike in the wild (on average 2 infants per litter). However, as females only have two nipples, they can only properly take care of 2 newborns. As a cooperative breeding species, the male and juveniles from previous litters also contribute to raising the young. However, this is not enough to compensate the limited milk production. There are several protocols describing ways to manage surplus infants (natural selection, bottle-feeding, euthanasia, etc). In this porject, we carried out a pilot study to optimize hand-feeding practices by rotating infants, before deploying a new decision-tree more widely. This protocol is a good example of the implementation of a 3R approach in a nonhuman primate breeding facility.

PC074

Achieving Environmental Enrichment in South Korea

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Abstract
Environmental enrichment (EE) promotes animal welfare and psychological well-being by providing sensory and motor stimulation, structure, resources, physical exercise, manipulative activities, and cognitive challenges that allow animals to express species-specific behaviors.

EE improves experimental results and decreases the number of animals used by reducing stress and anxiety, which can skew results. Many studies are underway to establish effective laboratory animal EE programs and demonstrate their beneficial effects. However, there is little statistically validated research on the factors influencing EE programs in laboratory animal research facilities.

Researchers and employees who care for and use laboratory animals in South Korea were surveyed to compare institutions that successfully and unsuccessfully implement EE in laboratory animals, identify success factors, and understand their need for ethical animal experimentation; 631 responses were analyzed. Respondents were divided into those who did ($n = 328$) and did not ($n = 303$) achieve EE. A binary logistic regression analysis was conducted to analyze factors related to EE achievements.

Individual perceptions, institutional and governmental support, and sociodemographic factors were analyzed for their impact on EE success. The need for a government-supported education program on laboratory animal research ethics was strongly felt by the EE achievement group. Financial support, having an attending veterinarian (AV) in the institution, and in-house training had significant effects on EE.

Institutional financial and government-supported education, AV employment, and training programs are needed to realize EE in laboratory animals. This study serves as a reference for designing EE programs in laboratory animal research institutions.

PC075

Vatinoxan Increases Medetomidine, Midazolam, and Fentanyl Concentrations in Rat Central Nervous Tissue

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Abstract
Vatinoxan is a peripherally acting alpha2-adrenergic antagonist that poorly penetrates the blood-brain barrier. In veterinary medicine, it is used to attenuate the adverse effects of medetomidine. Vatinoxan also appears to decrease the lead time to sedation after parenteral co-administration with alpha2-agonists, such as medetomidine. We investigated vatinoxan's impact on the concentrations of medetomidine, midazolam and fentanyl in central nervous system tissues after subcutaneous co-administration. Twelve healthy male Wistar rats (13–15 weeks old) received either medetomidine 0.25 mg/kg, midazolam 2 mg/kg, and fentanyl 0.01 mg/kg (MMF) or MMF combined with 5 mg/kg vatinoxan (MMF-V). Fifteen minutes later, the rats were humanely euthanized with intravenous pentobarbital. Plasma and samples of the left parietal cerebral cortex, thalamus, pons and lumbar spinal cord were analyzed for drug concentrations.

Treatments were compared using Bonferroni-corrected t-tests after one-way ANOVA. Cortical concentrations of medetomidine (144 ± 19.4 vs. 107 ± 13.1 ng/g, $p = 0.04$ (mean ± 95% confidence interval)) and fentanyl (2.3 ± 0.2 vs. 1.7 ± 0.3 ng/g, $p = 0.04$) were significantly higher in the MMF-V group. Cortex-to-plasma concentration ratios were also significantly higher for medetomidine (3.8 ± 0.7 vs. 8.0 ± 1.2), midazolam (2.0 ± 0.6 vs. 4.7 ± 0.7), and fentanyl (2.6 ± 0.5 vs. 5.6 ± 1.0) in MMF-V-treated rats ($p < 0.001$ for all drugs).

Vatinoxan increased early CNS drug exposure. The results support the 3R principles by refineming experimental animal sedation towards more reliable and effective medetomidine-based sedation protocols.

PC076

A Novel "Vaccine" Strategy Mitigates *Corynebacterium bovis*-associated Hyperkeratosis (CAH) in Athymic Nude Mice

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Abstract
A non-pathogenic Cb isolate (NPI Cb) and a skin microbiota component, *Corynebacterium amycolatum* (Ca) were evaluated as "vaccines" to determine whether they could protect against challenge with pathogenic Cb (PI). Crl:NU(NCr)-Foxn1^{nu} mice (N = 6/group) were randomized into 6 groups: 1. NPI Cb (10^8 CFU); 2. Ca (10^8 CFU); 3. and 4. NPI Cb or Ca followed by PI Cb (10^4 CFU) 2 weeks later; and 5. and 6. negative and positive controls receiving sterile media or PI Cb (104 CFU). Colonization was assessed biweekly via isolate-specific PCR assays. Skin lesions were scored 0 – 5 daily based on severity for 4 (Groups 1, 2, and 5) or 6 (Groups 3, 4, and 6) weeks. Skin biopsies were collected, and pathology scored at the study's termination. None of the mice receiving NPI Cb developed clinical signs nor was the PI detected. The mice receiving Ca developed a milder, delayed CAH after infection with the PI which was detected. The histopathology score for Group 3 was significantly lower than that of Group 6. Groups 4 and 6 had similar scores. These results led to the investigation of a practical vaccination strategy in which nude mice (N = 6/group) were housed on NPI Cb-contaminated bedding 7 or 3 days prior to PI Cb challenge. Mice exposed to Cb-free bedding served as controls. Only 1/12 vaccinated mice challenged with the PI developed CAH, whereas 6/6 unvaccinated challenged mice developed CAH. These findings highlight a promising strategy for mitigating CAH in nude mice.

PC077

Prefilter Test Matrix Placement Improves Pathogen Detection in Open Airflow IVC Racks

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Abstract
Real-time PCR testing of dust from the return air systems of open airflow IVC rack systems has provided a significant refinement in rodent colony health monitoring by delivering improved pathogen detection and replacing sentinel rodents for this purpose, consistent with the 3Rs. In this study, we compared real-time PCR pathogen detection of matrices placed to collect dust from the prefilter from two open airflow IVC racks over a 3-month period using the manufacturers' recommended collection devices as well as a high binding capacity matrix placed in front of the rack exhaust air prefilter. In IVC rack A, 14 viral, bacterial, or parasitic pathogens were detected using the manufacturer's device, whereas 19 pathogens were detected in the exhaust prefilter dust sample. Of the pathogens detected in both samples, the exhaust prefilter sample contained 4- to 71-fold more copies per PCR reaction. In IVC rack B, 6 viral, bacterial, or parasitic pathogens were detected using the manufacturer's device, whereas 15 pathogens were detected using the exhaust prefilter sample. Of the pathogens detected in both samples, the exhaust prefilter sample contained 22- to 96-fold more copies per PCR reaction. These data demonstrate the combined importance of the location and the material used to collect exhaust dust on diagnostic sensitivity and highlight that samples easily collected from the prefilter can provide improved mouse pathogen detection. The target audience for this presentation includes veterinarians, facility managers, and technical personnel who manage health monitoring programs.

PC078

Sentinel-free Soiled Bedding Rodent Health Monitoring Improves Pathogen Detection And Eliminates Sentinel Rodents

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Abstract
Reduction of the animal numbers used in research is a cornerstone of the 3R's principles and can be applied to today's animal health monitoring programs. Regular monitoring of rodent colonies for infectious agents is critical to maintain research colony health status and prevent inadvertent experimental variability. Sentinel-free soiled bedding (SFSB) is a rodent environmental health monitoring approach using PCR to detect rodent pathogens in collection material that has been exposed to soiled bedding from colony animals. Our experimental data show that over a 3-month health monitoring period of mice harboring rodent viral, bacterial and parasitic pathogens, SFSB detected 22 pathogens while soiled bedding sentinels detected only 10. These data, along with the peer reviewed literature, support the conclusions that SFSB provides improved pathogen detection compared to soiled bedding sentinels, eliminates the use of sentinel rodents for this purpose and can be used with any rodent caging system. Additional data on the frequency and method of exposing collection materials to soiled bedding will also be provided. The target audience includes veterinarians, facility managers, and technical personnel who manage colony health monitoring programs.

PC079

Adjustment of Anesthesia Protocol in Experiments with Pigs Anesthetized for More than 10 Hours

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Abstract
A good preclinical model must have minimal impact on animal welfare and on the parameters to be evaluated. However, pigs are known to have a high risk of malignant hyperthermia ("MH"). In this study, we aimed to propose an anesthetic protocol in a porcine model where anesthesia exceeds 10 h.

Fifteen pigs (*Sus scrofa domesticus*) weighing 39kg (35–45kg) were divided into three anesthesia groups: *inhalational sevoflurane (Protocol Sevoflurane: "PS"); **intravenous ketamine, diazepam and medetomidine perfusion (Protocol ketamine: "PK"); and ***Protocol "PK" with noradrenaline and glucose (Protocol "PKGN").

Hemodynamic and biochemical measurements were performed. Non-parametric tests were used to analyze our results.

Two out of five pigs from "PS" developed "MH". These "MH" pigs showed significantly higher maximum rectal temperature ($P = 0.04$), maximum expired CO2 ($P = 0.04$), maximum heart rate ("HR") ($P = 0.03$) compared to the other pigs. Also, "PS" pigs had a significantly lower minimum mean arterial pressure ("mAP") ($P = 0.03$) and it remains below 60 mmHg for longer ($P = 0.004$). In "Protocol PK", minimum glycemia was lower than other groups ($P = 0.01$).

Pigs anesthetized with sevoflurane developed "MH" when anesthesia lasted longer than 10 hours. The use of halogenated gases should be avoided in such cases. Ketamine (used in the "PK" and "PKGN" groups) prevented "MH" and achieved better "mPA" than the "PS" group. However, this protocol induced hypoglycemia in the "PK" group. Therefore, it was necessary to monitor and restore glycemia as seen in the "PKGN" group.

Thus, this study has allowed us to improve our protocol for prolonged anesthesia in pigs.

PC080

Building a Platform for Experimental Protocols and DOPs to Improve Animal Welfare and Science

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Abstract
Over the past two years, the Dutch Association for Laboratory Animal Science (DALAS) Working Group on Experimental Animal Protocols has developed an online national platform designed to facilitate knowledge sharing and improve experimental protocols. This initiative aims to enhance animal welfare and ensure more reliable research outcomes by evolving from rigid Standard Operating Procedures (SOPs) to more flexible and impactful systems: Animal (Dier in Dutch) Experimental Protocols (DEPs) and Direct Observation of Procedural Skills (DOPS).

From SOPs to DEPs: Flexibility and Impact: While SOPs provide standardization, they often fall short in addressing the nuanced needs of various animal models and procedures. DEPs offer the adaptability required to tailor approaches while fostering collaboration through the platform, enabling technicians and researchers to share best practices. The ultimate goal of this initiative is better care for animals and more consistent research data.

From DEPs to DOPS: Training and Assessment: DEPs form the foundation for DOPS—evaluation tools that connect protocols to hands-on training and skills assessment. These tools identify the minimum knowledge and competencies needed to perform procedures responsibly. To support professionals, the working group, in collaboration with Utrecht University of Applied Sciences, launched the *Assessor and Trainer in Laboratory Animal Science* course. This course equips participants to translate DEPs into DOPS, train and assess colleagues.

Looking ahead, the working group continues to advance DEP and DOPS development, supporting professionals via the online platform, practical training, and educational programs. Together, these efforts aim to elevate animal welfare and scientific robustness in experimental research.

PC081

Which Is the Best Animal Model to Study Osteoarthritis (OA)?

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Abstract
Osteoarthritis (OA) is a crippling disease. It affects more than 50% of the population over 50 years old. It has been studied for years using numerous animal models. The murine model, more specifically the mouse model, has been used extensively in an attempt to understand the pathophysiology of the disease, especially in genetically modified models. The rat has also been studied because of its ease of handling, availability, and its size. The rabbit is considered a large animal model; it was used several years ago, but it has been neglected for several reasons. The canine model is ethically a difficult model to use; the IACUC tend to reject protocols using dogs if an alternative is possible. The ovine model has become an interesting one but is tedious to use.

So, which then is the best model for OA studies? Easy question, easy answer: the best model is a human suffering from OA! In the meantime, we must concentrate on a specific question: What is the purpose of the study? Once that question is answered, it becomes easier to select an appropriate model. This lecture will present

several research proposals for which the thought process to select the best model for each proposal will be illustrated.

This lecture will review the most frequently used models in mice, rats, and dogs. We will also present a novel model, the MI-RAT.

PC082

The Use of New Technologies to Improve Laboratory Animal Welfare Monitoring

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Abstract
Continuous welfare monitoring is crucial in laboratory animals. It reduces wastage of resources caused by the generation of unreliable data due to stress related physiological changes. In most institutions it is difficult to implement continuous animal welfare checks since personnel cannot always be in the facility observing the animals. With rapid advances in technology, we envisage that technology can be used more effectively to enhance animal welfare monitoring.

The use of technology such as telemetry has proven to be reliable over time due to increased efficiency, repeatability, indefatigability and ability to effectively store data, while simultaneously eliminating human bias and inconsistency. This presentation focuses on the application of latest technologies such as artificial intelligence (AI) and how they can be used for continuous, non-invasive and accurate monitoring of animal health and behaviour. The presentation also explores the 3Rs with more emphasis on refinement, to investigate the gaps bridged by technology, the roles that should be performed by animal care professionals, as well as potential future uses of technology to enhance animal welfare monitoring.

Despite its effectiveness, the introduction of technology in animal research may cause challenges such as high initial setup cost and incompatibility with existing monitoring protocols. However, we recommend the use of existing technology and newly developed systems for environmental, behavioural and physiological monitoring as well as the prediction of animal welfare problems. By achieving this at higher intervals than humanly possible, technology can provide opportunities to amend protocols during studies and thus avoid attaining unreliable data.

PC083

Beagle Dog Quality of Life and Behavior Grid: A Tool for Experimental Ethology

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Abstract
Although there is a growing recognition of the importance of animal welfare, especially for companion animals (1), the behavioral needs of laboratory dogs have been relatively overlooked. Despite the fact that these animals often experience a variety of stressors, including those associated with experimental procedures, there is a paucity of validated tools to assess their behavioral well-being (2–3). This limitation hinders our ability to develop and implement effective stress-reduction strategies for laboratory dogs. To date, to the best of our knowledge, there are very few, if any tools for specifically assessing the behavior of domestic carnivores in experimental research settings in France. Therefore, we have studied animal welfare in healthy Beagle dogs using an assessment grid consisting of a section on the animal's quality of life, encompassing physical (hydration status, locomotion) and psychological (temperament, activity, social relationships) domains, as well as a section on behavior. The latter category was assessed through various tests involving the presentation of objects, unfamiliar individuals, and the presentation of an unfamiliar auditory stimulus within the dogs' home environment, and during a walk in a novel location to elicit a stress response. Scores were assigned to each item based on the experimenter's observations. We will discuss the preliminary results obtained using this grid, validated with a commonly used canine anxiolytic, and explore its potential applications in Beagle dog ethology

PC084

Training in Animal Experimentation: ComED's Initiatives in Distance Learning, Ethical Practices, and Mentorship Programs

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Abstract
ComED (Educative Commission) is an AFSTAL (French Association of Laboratory Animal Sciences and Techniques) commission (1) composed of scientific experts who work to improve training in animal experimentation. Their work encompasses developing training materials, promoting best practices, and supporting the recognition of training programs. They are also involved in discussions on the ethical use of animals in research and education.

During the last 4 years, ComED has organized 3 educational events related to initial and continuing education in animal experimentation. The first workshop (2021) examined the feasibility and relevance of distance learning in animal experimentation, addressing challenges posed by the era of Covid-2019. The second workshop (2023) focused on teaching methods for animal experimentation that do not involve the use of live animals. It highlighted alternative pedagogical approaches such as 3D models, virtual simulations, or instructional videos, drawing on feedback and experiences to assess the effectiveness of these methods. The third workshop (2024) centered on the implementation and evaluation of mentorship programs in animal experimentation. The discussions during this event notably stressed on the regulatory framework for mentorship and demonstrated how its implementation in France contributes to the continuous improvement of experimental practices and the responsible management of animal experimentation methods. The strengths and weaknesses of the programs and exchanges during these workshops, particularly in relation to the animal welfare and the application of the 3Rs principles, will be presented and discussed.

PC085

Digital Ventilated Cage Interlinking: Home-cage Monitoring System to Improve Welfare in Mouse Behavioural Testing

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Abstract

The Digital Ventilated Cage (DVC) system developed by Tecniplast S.p.A. has revolutionized animal housing in research facilities by allowing precise control of environmental conditions to ensure optimal animal welfare. Furthermore, the possibility of a long-term, 24/7 monitoring of animal behaviour directly in the home-cage, has led to uncovering new digital biomarkers in mouse disease models. In this study, the utility of DVC to further facilitate behavioural research is explored, focusing on cognitive, anxiety, and social tests in C57BL/6J mice.

Three tests traditionally performed out of the home cage (novel object recognition, light/dark, three-chamber tests) were adapted and executed in a new system called 'Interlinking', which consists of up to three DVC cages interconnected via tunnels through frontal cage openings. After habituation to the Interlinking tunnels, tests were carried out over three days. Experiments were conducted at CNR and IIT to ensure cross-lab comparison.

The results showed that this new system was promising particularly in the context of social behaviours. This was further supported by evidence of a close correlation between manual video scoring data and digital biomarkers extracted from DVC data, such as mouse activity and cage occupancy.

In conclusion, the potential of DVC Interlinking system is highlighted, creating novel experimental setups to improve animal welfare and accurate behavioural research. This study provides valuable insights into the integration of advanced housing technologies with classical behavioural methods, paving the way for more sophisticated and ethologically relevant experimental designs.

PC086

Long-term Biocontainment and Welfare in Water Buffaloes Enrolled for Experimental Research

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Abstract

A challenge trial was arranged in a BSL-3 facility to assess long-term efficacy of RB51-Brucella vaccine in pregnant water buffaloes (*Bubalus bubalis*). Eight water buffaloes (6 vaccinated and 2 controls) aging 3 years at 7–8 months pregnancy, were moved from conventional stables to a biocontainment unit, 10 days before challenge. There is no research available describing long-term detention of water buffalos in biocontainment conditions. This study reports infrastructure and managing approach applied until 6 weeks post-delivery and effects on animal welfare. Biocontainment stables with automatic ventilation were adapted to host buffaloes adding waterproof mats, dust-free sawdust bedding. Animals were placed in 4 stables hosting 2 individuals each. Rooms were interconnected as pairs by removable partition walls therefore, for four vaccinated animals it was possible to stable the animals altogether. Wall fixed brushes were applied as environmental enrichment. A camera system was available in each room allowing 24/7 monitoring. A specific diet was formulated as unifeed. Blood and hair samples were periodically collected to measure markers associated to stress. Body condition score remained optimal over the time. One control animal aborted 2 months after challenge due to Brucella infection, all the other animals delivered normally but in one case a dystocia required veterinary intervention. No behavioural changes were recorded in terms of maternal care or the nursing of newborn calves. These findings contribute to the EU-Partnership on Animal Health & Welfare and highlight the importance of integrating animal welfare practices in high-biosecurity facilities to optimize care, management and welfare of buffaloes.

PC087

Loss of Learned Lectures: A New Approach for Welfare Assessment for Rats in Experiments

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Abstract
Precise welfare assessment is essential for ethical and robust research, particularly in long-term studies and high-severity cancer models(1). Welfare assessments in laboratory animals remain critical, but combining various evaluation methods may give an idea of the stress in the respective animal. This study evaluates the potential of the performance of a trained behavior as sensitive, non-invasive indicator of stress and wellbeing in rats with hepatocellular carcinoma.

Six male Wistar rats, aged 28 days upon arrival, were trained to open a lockbox and climb onto a scale. After 21-days of training, hepatocellular carcinoma was induced by supplementing diethyl nitrosamine to drinking water for up to 122 days(2). Time to complete the behavioral tasks was recorded daily and compared with established welfare parameters, including hair and salivary corticosterone, salivary immunoglobulin A, and body weight.

Tumor development was insufficient and animal health and welfare was not compromised, this made testing our hypotheses impossible. Interestingly, we saw an impact of experimental procedures on stress markers and behavior.

Lockbox opening times correlated with the concentration of hair corticosterone, showing a triphasic pattern: high corticosterone and longer solving times initially, low corticosterone and shorter times mid-study, and rising corticosterone and longer times towards the end. However, for scale climbing the correlation was not consistent. Acute stress from a housing room change and magnetic resonance imaging under anaesthesia increased lockbox and climbing times, while repeated ultrasound under anaesthesia showed no distinct effect.

This study suggests trained behavior as a promising tool for welfare monitoring in animal experiments.

PC088

Improving Animal Welfare and Research Outcomes: Refining Orogastric and Intravenous Administration in Rats

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Abstract
Administration of substances to laboratory animals is a critical procedure that requires meticulous planning to ensure accurate delivery while minimizing potential adverse effects on animals. Despite its routine use in toxicological and pharmacokinetic studies, orogastric gavage and intravenous tail vein injection in Sprague-Dawley rats pose unique challenges that warrant refinement to reduce animal pain and distress. This study critically reviews current practices, considering the impact of test substance characteristics, vehicle choice, pH, volume, and duration of treatment, alongside the importance of operator training. By focusing on these factors, we propose evidence-based refinements to these techniques, integrating literature findings with practical recommendations for optimizing procedural protocols. Key insights include strategies to improve gavage precision and reducing risks of oesophageal injury, as well as techniques to enhance tail vein accessibility and minimize stress during intravenous injections. These refinements not only mitigate animal discomfort, but also improve emotional homeostasis, which is essential to maintain the validity of experimental models as well as the concept of well-being. Importantly, the findings underline the dual benefits of welfare improvements: enhancing quality and reproducibility of data and strengthening research robustness.

This work proposes novel procedural recommendations that address both ethical considerations and scientific design, providing a refined framework for toxicity and pharmacokinetic studies. By implementing these strategies, researchers can better align experimental outcomes with regulatory requirements while upholding the highest standards of animal welfare.

PC089

Reducing Weaning Stress in Mouse Offspring: The Benefits of Maintaining Familiar Environments

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Abstract
Weaning is a critical phase in animal care that often induces stress in offspring. Observable behaviors such as reduced food intake, withdrawal, and hiding are common for several days post-weaning. Stress during this period can impact welfare and

development. This study aimed to evaluate the impact of a novel weaning method on stress levels in rodent offspring, focusing on maintaining familiar environments for the weaned animals. Traditionally, offspring are moved to new cages during weaning. In this observational study, we implemented an alternative approach: instead of relocating the offspring, the parents were transferred to clean cages, leaving the offspring in their original cages with familiar housing, enrichment, food, water, and scents. Behavioral and physiological indicators of stress were monitored in the offspring before and after weaning. Offspring weaned using the novel method exhibited significantly reduced stress behaviors. Compared to traditional weaning, they displayed improved food intake, increased activity, and exploratory behavior, utilizing the entire cage for play and foraging. These findings suggest that staying in familiar surroundings helps alleviate the stress associated with weaning. Maintaining offspring in familiar environments during weaning minimizes stress, promoting better welfare and activity levels. This method can be easily implemented in animal facilities to enhance the well-being of laboratory animals.

PC090

Interrelation between Light Intensity and Activity of Mice in a Individually Ventilated Cage

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Abstract
The variability of light intensity within and between rodent cages distributed at different levels on racks has been known for a long time. This phenomenon has prompted the development of methods of systematic rotation of cages with the animals under experiment in order to expose all different groups of rodents to a homogeneous amount of light over time.

The aim of this study was to evaluate the impact of these differences in brightness within and between cages on the activity of mice both on the cage surface and in terms of voluntary wheel running compared with that of mice kept in identical cages but shielded from light because black tinted.

Activity monitoring was carried out using racks equipped with the DVC (Dynamic ventilated cages) system capable of recording the movements of the animals in the cage as well as the voluntary activity on the wheel in spatial-temporal terms.

Additional parameters such as body weight trends, water and feed consumption, intra-cage temperature and relative humidity were recorded and compared between the two groups.

The variations and differences in terms of brightness between cages and other environmental parameters have been related to the voluntary activity carried out by the mice, to their physiological condition and more generally to their state of well-being.

PC091

Comprehensive Management of *Corynebacterium Bovis* Outbreaks in Spf/Sopf Animal Facilities

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Abstract
Investigations into unexplained chronic skin lesions in BALB/c Nude mice led to the identification of the opportunistic bacterium *Corynebacterium bovis* in our animal facilities. *C.bovis* is known to be a pathogen in immunodeficient rodents used in biomedical research. It can cause clinical symptoms and potentially compromise scientific results. Its complete eradication from animal facilities is challenging and can only be achieved through rigorous measures.

Our management plan included the following strategies:

- **Enhanced Hygiene Program**: The hygiene protocols for animal husbandry, equipment, and personnel have been significantly reinforced.
- **Dedicated Quarantine Area**: A specific quarantine zone has been established for all suspect animal batches involved in high-value scientific studies.
- **Comprehensive Health Monitoring**: A thorough health monitoring program was initiated across all SOPF and SPF animal facilities to first determine the prevalence of *C. bovis* and subsequently track its decline in response to implemented measures. This health monitoring strategy includes quarterly environmental sampling and monthly PCR analyses of skin and/or feces from all housed animals.
- **Full Decontamination Protocol**: A complete decontamination of the facilities was executed, followed by a robust one-year monitoring plan for *C.bovis*.

The results of the one-year monitoring confirmed the *C.bovis-free* status of our animal facilities. Despite thorough investigations, the source of the contamination remains unidentified, with human origin being the predominant hypothesis.

To date, no scientific impact on research outcomes has been observed. Additionally, we will discuss the psychological and financial implications of managing such an outbreak crisis within the animal facilities.

PC092

Ceratonia siliqua in the Rat Osteoporosis Animal Model: Implementing a Culture of Care

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Abstract
This presentation exemplifies the most widely used animal model for investigating postmenopausal osteoporosis and the potential of *Ceratonia siliqua* (CS) as a non-pharmaceutical therapeutic alternative, by demonstrating a simple and easily implemented approach to safeguard animal welfare. The study complied with the European Directive 2010/63/EU, ensuring the highest animal care and welfare standards, PREPARE guidelines were taken into consideration and a wide range of humane endpoints were set. Thirty mature female Wistar rats were divided into: control, ovariectomized (OVX), and ovariectomized-plus-*C. siliqua* (OVX+CS). The rats were housed in stable groups and monitored daily for their health and well-being. Their body weight and food consumption were measured weekly. Prevention of possible animal discomfort was considered at each step, such as preemptive analgesia, heat-loss protection, protective lubricant eye ointment during anesthesia, etc. Bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA) at baseline, 3 and 6 months post-ovariectomy, indicated that the proximal tibial BMD was higher in the OVX+CS group compared to the OVX group at both 3 and 6 months. The *ex vivo* three-point bending test revealed a significantly higher thickness index in the OVX+CS group. These findings suggest that long-term administration of C. siliqua may serve as a viable non-pharmaceutical intervention to mitigate the severity of osteoporosis while maintaining high animal welfare standards in biomedical research.

PC093

Refining the Ovariectomized Rat Model for Postmenopausal Research: Enhancing Animal Welfare and Research Outcomes

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Abstract
Our Laboratory has extensively studied postmenopausal bone loss using the ovariectomized rat model, which closely represents post-menopausal women. Such animal studies are pivotal for successful clinical trials and subsequent complication-free management of osteoporosis.

Key points for improving animal welfare, reliability, and translational value, based on our publications, will be addressed, such as the animals' appropriate age; bone remodeling observed in female rats after 10 months, combined with post-ovariectomy bone loss, is ideal for studying osteoprotective substances. Welfare considerations include handling by experienced and continuously updated personnel with empathy, habituating the animals to the personnel and the operating room pre-operatively, application of refined surgical techniques and observant postoperative care, which minimize animal stress and potential complications. So is housing them together postoperatively in the same pre-operative groups, providing additional estrogen-free chow on the cage floor, long-nozzle water bottles, and incorporating appropriate environmental enrichment. Scientific recommendations of translational value include studying the proximal tibia separately, starting substance administration more than two weeks post-ovariectomy as per OECD guidelines, consideration of the appropriate study's duration (decreased trabecular bone volume is anticipated twelve weeks post-ovariectomy), the administered substance's pharmacokinetics, dosage, means and frequency of administration, and prevention of exposure to other estrogens (e.g., old polycarbonate and polysulfone cages). Confirming the absence of estrous by observing epithelial cells swabbed from rat vaginas is also scientifically advised.

Improving the ovariectomized rat model and prioritizing animal welfare, enhances both the animals' well-being and the model's translational relevance, contributing to developing more effective treatments for postmenopausal bone loss.

PC094

We are Just Warming Up: Non-invasive Monitoring of the Poikilothermy-endothermy Transition in Mouse Pups

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Abstract
Mice are born poikilothermic, relying on external heat from parents, littermates, or the environment until becoming fully endothermic. Identifying the transition from poikilothermy to endothermy can improve decisions on husbandry and animal care, while shedding light on thermoregulation in laboratory rodents, which can also inform wildlife research, in a context of climate changes.

Body temperature assessment in rodents has traditionally involved invasive methods, which cause stress and affect both animal welfare and temperature readout, due to stress-induced hyper- or hypothermia. We therefore applied a protocol for contactless temperature monitoring, using both thermal imaging and

subcutaneously implanted thermosensitive PIT-tags, read at a short distance. Thermal images are processed using in-house developed open-source software (ThermoLabAnimal), which automatically identifies individual animals, removes background noise, and calculates mean surface temperatures. Furthermore, we developed an in-house home-cage monitoring system capable of reading temperature from PIT-tags automatically. This approach reduces stress-induced variability, thus aligning with both the principle of Refinement and of Reduction, through better data accuracy and consistency. Cost-effective, user-friendly, and open-source, the system design and software will be made freely accessible, and can be applied to a range of studies involving body temperature monitoring, from thermophysiology to infection research.

Our results provide novel insights into the physiological mechanisms of thermoregulation and evolutionary adaptation, offering insight into current husbandry practices regarding thermal comfort for laboratory mouse pups.

PC095

Patch Perfect: Using Fentanyl Transdermal Patches for Pain Management in Göttingen Minipigs After Thoracotomy

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Abstract
Ensuring effective and consistent pain management is critical in surgical *in vivo* models in laboratory pigs, which have been increasingly used as translational models for drug development due to their anatomical and physiological similarities to humans. Proper analgesia not only promotes animal welfare but also minimizes stress and pain-induced variability that can compromise the reliability of scientific data.

In this study, we investigated the pharmacokinetics and efficacy of fentanyl transdermal patches for managing post-operative pain in female Göttingen Minipigs undergoing thoracotomy surgeries for the delivery of medicines via direct cardiac injection. Fentanyl patches were applied one day prior to surgery in the area behind the ears and replaced every 48 hours with a 4-hour overlap between patches for up to 7 days after surgery. Plasma samples were collected using central venous catheters at multiple timepoints, to determine fentanyl exposure levels, and the presence of post-surgical pain was assessed.

Results demonstrated that the 75 μg/h fentanyl transdermal patch generated plasma fentanyl concentrations consistently exceeding 0.3 ng/mL in female Göttingen minipigs. While species-specific fentanyl exposure guidelines for pigs are unavailable, these levels align with human therapeutic ranges, indicating adequate pain relief. Post-operative pain assessments confirmed the effectiveness of analgesia.

These findings highlight the utility of fentanyl transdermal patches as a practical and reliable method for post-surgical pain management in Göttingen minipigs in surgical models.

PC096

The Use of Floor Pens for Social Housing Naive Rabbits

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Abstract
In 2024, Inotiv RMS, created a new facility for social housing of New Zealand White rabbits. Current practice, in our breeding barrier, is to wean rabbits at five weeks of age into pairs or trios and maintain in cage housing until the point of sale.

The new facility allows rabbits to be housed in floor pens, with rabbits weaned directly into groups of forty rabbits. The pens have been designed to allow rabbits to interact with each other across a 60,000cm2 area. A variety of enrichment is provided. Rabbits remain in these social groups until the point of sale, which is generally between ten and sixteen weeks of age.

Whilst floor pens have gained popularity in research facilities in recent years, we present here their successful use by a commercial breeder. Rabbits are gregarious animals and are capable of forming complex social relationships with each other. Social housing has been shown to reduce stress-related behaviours.

Here, we present positive observations from technicians working in this facility, we have also received positive feedback from customers who have noted a calmer behaviour in the animals they have purchased. We have further plans for continuous improvements to rabbit welfare at Inotiv.

PC097

Improving Care and Welfare in Studies Using the Cerebral Open Flow Microperfusion in Rodents

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Abstract
Cerebral implants are crucial in studying neurodegenerative diseases, brain cancer, and other diseases. However, as such implants inevitably cause local damage to brain tissue and disrupt the blood-brain barrier (BBB), pharmacodynamics and pharmacokinetic results can be skewed by the implantation trauma and the ensuing leakage. Cerebral Open Flow Microperfusion (cOFM) is a long-term sampling technology that allows tissue trauma healing and BBB re-establishment after surgery. It thus makes it possible to collect samples from the brain interstitial fluid with an intact BBB. cOFM can be used in a wide range of species, but here we will present its application in rodents. Specialized husbandry and

handling is required to care for and maintain the health of animals with long-term brain implants. We therefore aimed to investigate the following animal care modifications: Additional animal care steps to maintain probe functionality between multiple study days through the use of the healing dummy, special observation and monitoring for infection at the cranial implant site, improved handling during cage changes to avoid undue stress to the implant, changes to standard animal housing as special enrichment to provide more vertical space for the implant, and special nestlet to avoid disturbing the implant and continuous sampling. Implementing these animal care modifications can make it possible to study disease progression and treatment over a long period of up to 40 days, as well as providing the potential of performing several sampling days within a study with the same animal.

PC098

Rabbit Welfare Working Group: Building Consensus and Collaboration for Program Improvement

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Abstract
Rabbits are an important species for developmental toxicology research, often being used as the primary non-rodent species. As work with primates and dogs becomes more restricted, work with rabbits will likely increase due to ease of breeding, management, and availability. As work with rabbits expands, further consideration is needed for their behavioral management, pain recognition and mitigation, procedural refinements, welfare assessment, and euthanasia. In recognition of the 2023 Lunar Year of the Rabbit, a Rabbit Welfare Working Group was formed to develop best practice recommendations for rabbits in a research setting. The group consisted of subject matter experts based in breeding, discovery, and safety assessment business units with interest in topics related to rabbit care and management, grouped into sub-committees based on specific topics including basic behavioral needs and housing, social housing, low stress handling and restraint, routine animal care practices, animal welfare assessment, pain management and humane interventions, and euthanasia. The working group members met virtually in 2023 and 2024, with a Rabbit 3Rs Workshop held in 2024 for those external to the working group. The group worked to achieve consensus on best practices, developing 12 recommendations that were circulated to corporate leadership to garner support. The recommendations will be used to develop a rabbit welfare assessment tool to permit objective evaluation and allow sites to identify priorities and set goals to work towards to improve rabbit welfare.

PC099

Effects of Diabetes Mellitus on Mandibular Growth in Rats with Different Masticatory Force Loading

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Abstract
Type I diabetes is a metabolic disorder affecting bone metabolism. One-month-old male Wistar rats were studied for the effect of diabetes on mandibular growth, while the effect of consuming hard or soft food on mandibular growth was also assessed for 30 days. The study complied with the European Directive 2010/63/EU, ensuring the highest animal care and welfare standards, and was licensed following protocol evaluation. The rats were divided into 4 equal groups of 7 animals each: diabetic eating hard food (pelleted diet), diabetic eating soft food (powdered diet), control eating hard food, and control eating soft food. They were housed in open-top cages during the 30 days, monitored daily by the animal caretaker, weighed twice weekly to ensure food consumption and growth were normal, supervised by the laboratory-designated veterinarian, and were provided environmental enrichment tailored to ensure their welfare and reduce stress. At the beginning and the end of the study, head cone-beam computed tomographies (CBCT) were taken under brief sedation, while histological analysis was performed at the end of the 30 days after euthanasia. Preliminary results in the analysis of the CBCTs showed on the one hand that diabetes affects the development of the mandible, and on the other hand that the consumption of soft food, in diabetic and non-diabetic animals, further limits the development of the mandible. Definitive conclusions with levels of significance will be drawn after completing all analyses of CBCT and histology. These conclusions will have a translational value to the clinical condition.

PC100

Strategies for Improving three Rs in a Swiss Medium Size Rodent Facility

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Abstract
The Center of Phenogenomics (CPG) is a centralized animal facility serving the scientific community of EPFL (Lausanne's Swiss

Federal Institute of Technology). This centralization represents an asset to monitor animal research methods and procedures before, during and after the experiment, and thus ensure best practices for the entire time animals stay on our premises. Here, we will discuss which approaches have been implemented at different life stages of the animals to fully embrace three Rs concept and Culture of Care.

In particular, we will present the implementation of transparent plastic tunnels as enrichment and tools for gentle handling in mouse ventilated cages. At first, a test was conducted to define the preference of mice between red or transparent tunnels, either placed on the floor or suspended from the upper grid. From our observations, we opted for transparent clip-on plastic tunnels attached to the grid, which have been deployed throughout the entire facility. A follow-up opinion survey of all caretakers and technicians was conducted to assess acceptance.

In addition, we will also discuss programs implemented to optimize sharing of animals or organs between EPFL researchers, which have allowed over the last two years the exchange of over 450 mice and 300 organs.

Finally, we will also present the programs in place at the end of the experiment, either to rehome rats in private households or to donate rodent carcasses to feed carnivores at a care center for injured wild animals.

PC101

Corticosterone Measurement in Hair as an Early Indicator of Chronic Stress in Laboratory Mice

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Abstract
Mice, the most widely used species in animal experimentation, pose a significant challenge for welfare assessment. As prey animals, they conceal pain and distress in the presence of humans, complicating the evaluation of their well-being. Corticosterone, the primary glucocorticoid in mice, reflects stress levels and is traditionally measured through invasive blood sampling (Elmi et al., 2020). Alternative matrices such as feces, urine, and, more recently, hair offer non-invasive approaches (Ataallahi et al., 2022). In combination with environmental and behavioral assessments, corticosterone levels provide valuable insights into laboratory mice's welfare (Veit & Browning, 2021).

This study evaluated the effects of moderate to severe chronic stress on seven females NOD-Prkdcem26Cd52Il2rgem26Cd22/NjuCrl immunodeficient NCG mice subjected to daily 30-minute restraint sessions. Health status, weight, behavioral responses to anhedonia and anxiety tests, and corticosterone concentrations in urine, feces, and hair were measured and compared to a control group of seven mice. The animals, part of a humanization procedure that did not yield satisfactory levels of human immune cells, showed no clinical or behavioral signs of stress. Behavioral tests revealed no significant effects of chronic stress, which was reclassified a posteriori as mild to moderate.

However, subtle differences emerged: stressed mice exhibited reduced body weight and elevated corticosterone levels exclusively in hair samples. These findings highlight the utility of non-invasive corticosterone measurement in detecting early welfare alterations, undetectable through traditional methods. This approach can enhance welfare assessment, improve pain management, and inform enrichment and training strategies for laboratory animals.

PC102

"Time for Recess!": A Novel Approach to Enhancing Rat Welfare Through Cage Enrichment

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Abstract
Standard environmental enrichment for laboratory rats housed long-term often proves insufficient, leading to stress-related behaviours like hypoactivity, alopecia, and porphyrin secretion. This case study aimed to improve cage enrichment within a specific rodent facility by introducing the "Recess", a multi-perforated plastic semi-tunnel-like structure designed to encourage species-specific behaviours.

A preliminary study tested the Recess on the cage floor in two configurations: horizontal and vertical (N = 18 rats, 2–13 months old, both sexes). Behavioural observations were conducted via scan sampling over multiple daily periods across one week.

Results showed that rats spent significantly more time interacting with the horizontal Recess (18%) compared to vertical (3%). Furthermore, rats spent 11% of their time interacting with the Recess versus only 4% with other existing enrichments like tunnels, cellulose bags, sizzle, and wooden blocks. The Recess promoted resting, social interactions, exploration, and physical activity, appearing adaptable to different life stages. Despite promising results, the small sample size and lack of inferential statistical analysis limit the robustness of the findings.

Recent trials demonstrated that attaching the Recess to the cage lid is feasible without compromising cage ventilation, suggesting another position worth exploring. Plans to develop 3D printing prototypes and scaling trials with larger cohorts and statistical analysis to assess long-term durability, usage, and feasibility are underway.

This study provides a promising foundation for improving enrichment practices, with potential to significantly enhance laboratory rat welfare, highlighting the importance of creative solutions adapted to individual needs, paving the way for more personalized approaches in animal welfare.

PC103

Material or Habit? A Study of Bottle Preferences in Rodents

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Abstract
To improve the welfare of laboratory animals, we aimed to investigate whether rodents exhibit a preference for drinking from bottles made of different materials.

Sterilized and unsterilized bottles made of three different materials (two types of plastic and one of glass) were tested on Wistar rats and C57BL/6 mice of both sexes, which had been previously habituated to have a plastic bottle positioned on the right side of the cage.

To assess bottle preference, the animals were housed in cages that allowed for the simultaneous placement of two bottles, one on each side, and water consumption was measured. Various combinations of bottles were evaluated over a period of six weeks, with the positions of the bottles alternated between right and left each week.

The results from both groups of rodents indicated a higher water consumption from the bottle located on the right side of the cage, regardless of its material or whether it was sterilized or not.

Based on these findings, we sought to determine whether this choice was influenced by parental teaching or learned through habit. To address this question, we conducted the same experiment with several groups of juvenile rats that had no prior habit regarding bottle positioning. The results showed that bottle choice is significantly influenced by what the juveniles learn from their parents from birth to weaning.

PC104

Bedding Preference. Let's Ask the Mice!

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Abstract
Previous studies have analysed the physicochemical characteristics of four common bedding materials used in animal facilities (corn, cellulose pellets, and two types of poplar wood).

In this preference study, we wanted to determine which bedding would be chosen by mice for their daily activities.

To achieve this, we housed a population of C57BL/6 strain mice (both males and females) in two connected cages, each containing different bedding, under identical environmental conditions to allow for free movement.

Various bedding combinations were created to assess all possible comparisons (a total of twelve).

Data on food and water consumption, as well as excrement production, were collected weekly for each cage, and the location of the nest and enrichment items were recorded daily.

The results revealed higher food and water consumption with poplar wood bedding, but greater excrement deposition with corn and cellulose pellet bedding.

Additionally, the mice showed a clear preference for poplar wood bedding for nest and enrichment locations, over corn and cellulose pellet bedding.

PC105

Can Scented Gnaw Sticks Provide Better Environmental Enrichment to Single-House Mice?

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Abstract
A 3R study was conducted to improve environmental enrichment particularly for single-housed male mice in the breeding unit. The study investigated the impact of scent additives on the utilization of gnaw sticks aiming to determine how various scents influenced gnawing behaviour and whether these scents could improve environmental enrichment.

In the study, single-housed male mice were provided with a weighted gnaw stick treated with one of ten different essences (e.g., nougat, blueberry, hazelnut) or a standard unscented gnaw stick. Each mouse retained its assigned stick for two weeks. The assessment involved weighing the used stick and calculating the weight difference from its initial weight to evaluate the effect of the scent on gnawing behaviour. The results indicated that the mice preferred certain scents over others, and that the mice preferred scented sticks over the unscented ones, that were not utilized by the males at all.

PC106

Health Screening - Going Animal Free

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Abstract
Being fully committed to the 3Rs the University of Aberdeen invested into a review of using live animals for routine health screening.

Different methods using no or less animals in health screening were compared. It was decided to trial an adapted shake cage method using specific collection media called a Ghost sentinel® offered by FERA Science.

Two trials were carried out, comparing our current soiled bedding sentinel method with the Ghost Sentinel® method. Samples were taken from open cages and IVCs and were analysed in 2 different laboratories comparing results of a very comprehensive list of pathogens as recommended by FELASA.

All agents reported using sentinels were also detected in the Ghost sentinels®. The trials also showed that the more direct method of the ghost sentinel® detected pathogens more consistently due to the increased sensitivity. A new pathogen was also detected by the Ghost Sentinel® which had not previously been reported using the sentinels.

The presentation will discuss these results in detail. It will also discuss how the Ghost sentinel® works, what other testing has been carried out since the initial trials and feedback from the technicians who perform the shaking of the cages.

These trials demonstrated that using molecular detection and a more direct testing method allows for improved sensitivity of

detecting pathogens. In addition to that, this system is fully supporting the 3Rs, in particular replacement of animals.

The University of Aberdeen has used this new screening system since January 2024 with great success.

PC107

Water Activation Multicomponent System (WAMS) for Animal Facilities

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Abstract
Water activation multicomponent system (WAMS) has been designed and the pilot model of the WAMS underwent rigorous testing. The system efficiently processes liquids with a capacity of 0.5 to 500 m^3 per hour, depending on its size. Additionally, the safety and biocidal effects of WAMS-processed water were studied on 20 Wistar rats randomly divided in 2 groups - 10 animals in each. Animals were provided standard laboratory rodent diet 5001 and water (clean tap water in control and WAMS -processed water in test group, respectively) ad libitum for three months. CNS assessment by Irwin test, body weight monitoring and stool analysis were performed weekly. Finally, the animals were euthanized, gross necropsy and histopathological examination, as well as microbiological analysis of feces, was conducted. Research protocol was approved by the Ethics Committee of TSU Aleksander Natishvili Institute of Morphology (approval №025 12/12/2023). The study revealed: No differences in the behavior of the animals from various groups; No alterations in the microscopic structure of the internal organs; Compared to the animals in the control group, those receiving WAMS-processed water exhibited an average weight gain of approximately 80+/-10 grams; Interestingly, microbiological stool analysis revealed no differences between the groups, which is particularly significant given the bactericidal effect of the water activated by the WAMS; Given its stable biocidal properties and observed health benefits, the WAMS has significant potential for practical applications in maintaining water quality and promoting the well-being of laboratory animals in animal facilities.

PC108

Non-Invasive Method for Mouse Genotyping: Combining Ethics and Collaboration Animal Facility/Research Teams

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Abstract
In response to ethical and regulatory challenges, developing non-invasive methods for mouse genotyping has become a priority. In 2021, a project was initiated within an IBDM team to replace tail biopsies with a technique based on DNA extraction from mouse hair for PCR-based genotyping.

Through close collaboration between the animal facility and research teams, IBDM successfully adopted the method in 2023. This project, aligned with European recommendations, improves animal welfares and simplifies administrative procedures.

This initiative highlights how scientific curiosity, ethical commitment, and collaboration between animal facility and research teams can reconcile experimental efficiency with adherence to ethical standards.

A dual-presenter format will detail this collective project: one presenter will explain the laboratory optimization of DNA extraction from mouse hair, while the facility manager will describe its implementation in the animal facility and its adoption by IBDM research teams.

PC109

The Impact of Ultrasonic Noise on Laboratory Animals

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Abstract
Every organism experiences the world around it in a unique way – a consequence of the distinct differences in sensory capabilities observed throughout the animal kingdom. With modern advances in technology, it is possible to investigate the impacts that sounds beyond the human hearing range may have on various organisms. Rodents comprise the most commonly utilized animal in research and like many other organisms have a much broader hearing capacity than humans. The hearing range for rodents is ~250 Hz to 80 kHz; not only are rodents able to hear higher frequency sounds, but they use these ultrasonic frequencies to communicate. Unfortunately, it has been determined that the laboratory environment is rife with ultrasonic noise which may not only be a source of stress for laboratory rodents but also inhibit their ability to communicate with one another. As a means of better understanding the significance of ultrasonic noise within the laboratory, rats were either exposed to no noise, broadband white noise, or ultrasonic noise for 14 days. Following exposure, rat behaviour and physiology were assessed through classical tests of anxiety-like behavior, body temperature, fecal corticosterone, and adrenal gland weight. Rats exposed to ultrasonic noise exhibited a distinct behavioral and physiological profile, suggesting ultrasonic noise is not innocuous. A thorough understanding of the impacts of ultrasonic noise on rodents will lead to improved animal care and welfare as well as improve the validity of rodent research.

PC110

Humane Endpoints in Mouse Pups under 5 Days of Age

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Abstract
This presentation will share our bespoke neonatal mouse health monitoring program designed to enhance animal welfare in neonatal mouse studies by enabling the early identification and removal of mouse pups at risk of mortality, thereby reducing suffering. Neonatal mice are particularly vulnerable during the first few days of life, making early monitoring and timely intervention critical for their welfare.

The program employed a combination of behavioural and physical assessment of mouse pups in the first few days of life after inoculation to detect signs of health deterioration. Key indicators included lack of milk spot, loss of righting reflex, and general poor appearance. A trained team of technicians conducted daily health checks, utilizing visual observations with two hour follow ups for at risk animals to ensure comprehensive assessments.

Upon poor health indicators at follow up health checks, immediate action was taken to remove them from the study. This proactive approach not only minimized suffering but also provided an opportunity for further investigation into the underlying causes of neonatal mortality.

Data collected over a 12-month period demonstrated a significant reduction in the incidence of suffering among neonatal mice, with a marked decrease in the number of pups assumed "cannibalized by dams". This program serves as a model for implementing similar welfare-focused strategies in other research settings, emphasizing the importance of early intervention in mitigating suffering and enhancing the overall ethical standards of animal research.

PC111

Serum Buprenorphine in Mice after Dietary Dosing

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[2]*University of Oslo, Oslo, Norway*

Abstract
Consistent serum concentrations of analgesics is the basis for efficacious analgesia in animal models. We have assessed daily intake of SDS RM3(E) pellets with/without added buprenorphine and measured the serum concentrations in groups of 12 wk old male and female C57BL/6NRj mice (n = 4) after dietary dosing for 72 h. The aim of the study was to document a methodology of dietary dosing that achieves stable and clinically effective serum buprenorphine levels in mice (~1 mg/ml) housed in groups of four or single housed.

1. Body weight and daily diet consumption (1.3–1.6 g/10g body weight) was unaffected by buprenorphine in the diet at concentrations of 4, 7 and 16 μg/g food pellet. Diet consumption correlated with a buprenorphine intake of 0.7, 1.0 and 1.8 mg/kg/24 h. Serum concentrations of buprenorphine were all at or above clinically effective concentration and demonstrated a dose-response correlation at 24 h and 48 h after start dosing, and reducing concentrations at 6 h and 12 h after stopping dietary dosing.

2. When the buprenorphine concentration was adjusted to 2.5 μg/g food pellet, body weight and daily diet consumption (1.2–1.7 g/10g body weight) was again unaffected. Diet consumption in female and male mice correlated with a buprenorphine intake of 0.4 and 0.3 mg/kg/24 h, respectively. Mean buprenorphine serum concentrations were 0.8–3.0 ng/ml at 18 h, 22 h, 24 h, 28 h, 34 h, 44 h, 50 h and 62 h after start dosing, and showed reducing concentrations at 6 h, 12 h, 18 h and 24 h after stopping dietary dosing. The methodology of dietary dosing is suitable for clinical studies in surgical mouse models (ongoing).

PC112

Colony Managers in the Netherlands

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Abstract
Animal breeding and colony management are crucial components of many animal laboratories. By combining knowledge and expertise in the Netherlands, we have identified numerous opportunities for refinement and reduction in this field. Therefore, a group of colony managers has organized within the Dutch Association for Laboratory Animal Science to improve animal breeding.

Our colony managers group aims to share knowledge and experiences related to the breeding of various species. We provide advice on breeding strategies and share insights on maintaining different genetically modified animal models. To achieve this, we organize meetings and keep the group informed about changes in laws and regulations. We also share standardized forms and SOPs for common requirements across the country, such as GMO passports.

Additionally, we participate in various meetings with other working groups in the laboratory animal field, including the Netherlands Food and Consumer Product Safety Authority (NVWA), the Ministry of Agriculture, Fisheries, Food Security and Nature, and the Netherlands National Committee for the protection of animals used for scientific purposes (NCad). Additionaly, we are promoting breeding practices that can increase efficiency and hopefully reduce the number of unused animals.Through these efforts, we have developed into a committee capable of advising professionals on colony management and the breeding of laboratory animals.

PC113

Advancing Canine Welfare Monitoring: Integrating Machine Learning for Personalised Behavioural Analysis

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Abstract
Whilst there have been significant advances in behavioural analysis techniques and home-cage monitoring tools for rodents, comparatively fewer developments have focused on improving the monitoring of wellbeing in other species.

In dogs, many widely used welfare monitoring approaches rely on qualitative assessments and scoring systems. However, many of the advanced techniques employed in rodent studies could be relatively easily adapted for other species. In our study, we implement state-of-the-art deep learning and machine learning-based tracking and behavioural analysis in the dogs housed in our kennels. This will allow us comparing current gold-standard welfare monitoring methods for dogs with newer, more advanced approaches. These innovations are expected to enhance the sensitivity and specificity of behavioural analysis for undisturbed observational studies, facilitating the evaluation of animal welfare within the unique UZH research beagle colony at the Vetsuisse Faculty of the University of Zurich.

The substantial improvements in data quality provided by these advanced methods will enable the adoption of personalised medicine approaches similar to those being developed for humans. By establishing personalised behavioural profiles for dogs, researchers and animal caretakers will be able to objectively assess behaviour (undisturbed and continuously over 24 hours), thereby increasing the sensitivity and specificity of detecting individual behavioural changes. Personalised behavioural profiles have the potential to replace the current gold standard of general scoring systems. Furthermore, this project could contribute to the development of enhanced welfare monitoring systems applicable to a broader range of species.

PC114

Pride and Prejudice – the True Story of a Rodent Low Stress Handling Implementation

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Abstract
When leading a major change project, who hasn't heard these rebuttals at least once? Who hasn't had to deal with reluctance to reshape deep rooted habits and fear of routine upheaval? We weren't the exception when we tried to implement low stress handling (LSH) in our large-scale breeding facilities. LSH is widely documented by the scientific community as a true welfare improvement for rodents. But is it low stress for the people handling the mice? Literature is overflowing with quality scientific work proving the need for this change. Yet, when it comes to implementing these methods and reaching buy-in from stakeholders in a lasting way, it can quickly become a lonely and winding road. Sharing mistakes and how we can learn from them is as critical as sharing successes, and this presentation will offer practical examples on how to navigate animal welfare change management. It will explain how we planned this project confidently, the walls and failures we have encountered. We managed to overcome them by taking it back to the beginning. We had to deconstruct preconceived ideas (ours included), commit the top management to act, and communicate actively and frequently to all layers of our institution. Only then we successfully guided the change and reached long lasting implementation.

PC115

Changing Rodent Formula in Rodent Breeding Establishments: Why and How?

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Abstract
The field of animal nutrition has evolved significantly, and so too should the formulas used in rodent breeding. A team of stakeholders and subject matter experts, including external consultant, collaborated closely with laboratory feed manufacturers in Europe and North America to develop a fixed diet formula that could be standardized worldwide while meeting the needs of researchers. Six potential rodent diets, varying in protein and fat content, were developed with test batches manufactured in both the US and Europe. These diets were evaluated using five different rodent strains (mice and rats).

The studies were structured as follows: Initial 12-Week Growth Study: Monitoring growth, food intake, and overall health in both sexes of multiple strains, Two-Generation Breeding Study: Assessing the diet's impact on breeding success, pup numbers, and weaning weight and Follow-Up Growth Study: 12-week growth study in outbred strains to evaluate longer-term effects and consistency across different manufacturers.

Following thorough testing and validation, a diet containing 19% protein and 6% fat was chosen as the new FRD(B) diet formula. This diet met all the project team's goals, including maintaining phenotype consistency and ensuring the ability to be shipped internationally with minimal logistical challenges.

This presentation aims to shed light on the process behind selecting, comparing, testing, and validating the new rodent breeding formula, underscoring the importance of scientific collaboration and rigorous testing in driving improvements in animal model nutrition.

PC116

Something to Hang on To! A Cage Ladder for Climbing and Handling

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Abstract
At Lund University laboratory animal facility a cage ladder was designed for mice as a climbing structure. The need for this device arose when a new caging system was put into use; Innovive IVC single use cages, that didn't have a cage bar that the mice could climb on. The climbing structure provides the ability for mice to climb and exercise and also a third dimension of the cage. Beyond that it can be used as a handling device. In a study it was shown that, like tunnels, using home cage ladders to pick up mice reduces anxiety and avoids the aversion that is induced by picking up mice by their tails. The ladder can be used as both enrichment and handling device in any mouse cage. It is easy to pick up the mice with for transfer and inspection of the mice

including the ventral side. The mice can cling to it making them feel secure.

PC117

Mouse Cage Grid Cleanliness Evaluation Extending Change Intervals from 4 to 8 Weeks

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Abstract
We have evaluated the possibility of extending the mouse cage grid and lid change interval from 4 to 8 weeks. By extending the interval the AST Got (Animal Sciences and Technologies, Gothenburg) department will be able to shift hours spent on grid change to animal husbandry and welfare work, providing more study support to TAs and increase our investigative safety study load. Expenses and environmental impact will be reduced due to less washing and autoclaving as well as the heavy physical workload for staff. Less frequent changes will potentially reduce stress for the animals, leading to better science. The study was conducted in collaboration with an external company over 10 weeks, sampling a total of 125 cages, comprised of either IVC or static cages with group or single housed males or group housed females. Bacterial growth has been assessed by using TPC pressure plates, and for evaluating general cleanliness, ATP levels were measured with a luminometer. Relevant cut-off values have been set for each assessment after extensive literature reviews. Statistical evaluation shows no biologically relevant variation between week 2 and 8, and results fall far under the thresholds, so the conclusion is that the change of grids and lids can be made every 8th week instead of every 4th week.

PC118

Definition of Species-specific Needs (DoSSN) for Physiological, Social, and Mental Health

F. Christian Pipp[1], S. Sheridan[2], A. Aricó[3], C. Quigley[4], J. Coenen[1], K. Caspersen[5], J. Holschbach[1], P. Empting[1], H. Elhawi[6], R. Nahoom[6], C. Ciampolillo[3], A. Schneider[1], B. Alisantosa[7], S. Silveira[1], M. Whiteman[7], D. Elbirt[8], K. Kleinschmidt-Dörr[1] and Merck Heads of Vivariums and Merck Attending Veterinarians
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Abstract
Globally, there are significant disparities in laboratory animal housing and care standards, which have historically guided the design of animal facilities. In 2019, Tom Beauchamp and David De Grazia published "The 6 Principles of Animal Research Ethics," establishing species-specific basic needs as a guiding principle for animal care. The World Health Organization (WHO) defines health as a state of complete physical, social, and mental well-being. To ensure that data derived from animals are representative of humans, we propose applying this health definition to animal housing conditions.

Our guiding principle for laboratory animal care is to meet the species-specific needs aligned with this health definition. We began by collecting and evaluating information on the behaviors of various species, followed by an assessment of the conditions necessary to fulfill these behaviors. Consequently, we developed a comprehensive list of Definitions of Species-Specific Needs (DoSSN) for species including mice, rats, rabbits, zebrafish, guinea pigs, dogs, mini pigs, and non-human primates. This list serves as a framework for refining our animal care programs, planning housing systems, and guiding investments across our facilities.

Drafts created in collaboration with veterinary teams were reviewed to ensure that solutions address animal welfare, practical considerations, and scientific requirements. Our goal is to establish this methodology to fulfill as many needs of our animals for their holistic health as possible while identifying limitations, such as the inability to provide free partner choice or unrestricted exploration.

PC119

Determination of Murine Colony Weaning Rate by Using Laboratory Animal Breeding Software Mosaic Vivarium

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Abstract
The ability to effectively manage mouse colonies, especially with regard to breeding, is increasingly important. Careful calculation of the number of breedings in order to produce the number of required animals is essential under scientific and animal welfare aspects. The colony production rate (or colony weaning rate – CWR) is defined as the number of animals weaned per breeding female and week and represents the most important reproductive efficiency parameter of a breed and therefore is often required for careful breeding planning. In spite of the urgent need of the CWR parameter to characterize reproductive performance of murine strains in breeding calculations there are only few publications listing this parameter for various strains. Due to these missig data, retrospective breeding data of a specific colony can be used for CWR calculation and then be utilized for prospective breeding calculations for this strain. We present here useful equations, computational details, software requirements, and record-keeping responsibilities necessary to calculate the CWR in the Mosaic Vivarium animal husbandry software. By using the potential pregnancy time for determination of breeding duration, we can

unproblematically implement also non-littering females into electronic CWR calculation resulting in actual current values without any exclusions. We further describe useful algorithms for implementation of report start and end boundaries for CWR calculation. Futhermore, we established a diagnostic module which proved as a valuable tool not only for debugging initial implementations of the algorithm, but also for identifying data entry errors or omissions.

PC120

Strategies for Optimization of Peri-Operative Care of Pigs

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Abstract
Pigs are used for various biomedical research areas because of their similarity to humans in size, physiology, and anatomy. This often involves some kind of surgical procedure. Providing good peri-operative care is essential for a successful research outcome and for animal welfare. The peri-operative care includes preparation, induction, recovery and appropriate anesthetic and analgesic management. Moreover, socialization and training are also important factors for good peri-operative care, as they help the animals to interact with technicians and build trust before the surgery. In this presentation, we will share our best practices and experience in providing optimal perioperative care for minipigs at Novo Nordisk.

In addition to socialization and training, we have focused on maintaining body temperature, recovery pens and peri-operative analgesia, and creating a dedicated team of technicians who are responsible for the anesthesia procedure in collaboration with veterinarians. We will show the methods we use to keep the body temperature of minipigs stable during anesthesia and surgery, our perioperative analgesic protocols, and our methods to ease recovery when the minipigs return to the stable. Lastly, we will discuss how we ensure that the optimized perioperative care and improved animal welfare are consistent across different research groups and projects.

PC121

Long Term Inhalation Anesthesia with Isoflurane in Sprague Dawley Rats for a Microsurgery Course

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Abstract
Long-term inhalation anesthesia with isoflurane in Sprague-Dawley rats has been established as a dependable and effective approach for microsurgical training, particularly for procedures involving the femoral artery and vein. This study aimed to evaluate and validate a protocol for extended anesthesia tailored to microsurgery courses. A total of 32 Sprague-Dawley rats were anesthetized using isoflurane vaporized in a mixture of N_2O and O_2, with body temperature maintained at 37°C and fluid balance preserved through subcutaneous administration of warmed Ringer's acetate. The protocol enabled the simultaneous anesthetic maintenance of two animals for up to 7 hours, with a success rate of 75%. Among the successful cases, 11 rats completed the full 7-hour duration without complications, while 13 were maintained for shorter periods. Key benefits of the protocol included rapid induction, precise control of anesthesia depth through continuous monitoring of vital parameters, and minimal complications. No significant respiratory or circulatory disturbances were observed, and respiratory rate and SpO_2 levels remained stable throughout. This method underscores the suitability of isoflurane inhalation anesthesia for prolonged procedures requiring stable conditions and minimal physiological impact, making it highly applicable for training in advanced surgical techniques.

PC122

Determination of the Ammonia Concentration and Change Frequency of the B6.Cg-Tg(K18-ACE2)2Prlmn/J Mice

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Abstract
Mice cage bedding plays a fundamental role in communication and social interaction, providing suitable conditions for waste absorption, nesting, temperature maintenance, and the preservation of natural odors. Bedding also affects ammonia concentration, serving as a factor for determining replacement intervals. However, there are still no established guidelines regarding exposure limits for rodents. This study aimed to compare different bedding substrates to determine the most suitable type of bedding for B6.Cg-Tg(K18-ACE2)2Prlmn/J mice, focusing on replacement frequency and revising the routine of the Rodents and Lagomorphs Breeding Service (SCRL)/Fiocruz. In Study 1, ammonia concentration was measured in 80 IVC cages after bedding replacement (standard management: 7 animals per cage, *Pinus elliottii* wood shavings, and weekly bedding replacement), determining a cutoff point of 130 ppm. In Study 2, 10 IVC cages (7 animals per cage) were used with three different types of bedding substrates: *Pinus elliottii* wood shavings, *Populus tremuloides* Aspen shavings, and *Pinus elliottii* flakes. Measurements were taken on Mondays, Wednesdays, and Fridays from the third to the eighth week of the animals' life. Bedding replacement occurred when the ammonia concentration reached the cutoff point established in the first study. In this study, a difference was observed in the replacement interval between cages with *Pinus* wood shavings (7.47±3.54 days) and those with *Pinus* flakes (16.97±6.23 days), while Aspen showed an intermediate interval (12.7±8.09 days). Therefore, the

use of Pinus flakes for managing B6.Cg-Tg(K18-ACE2)2Prlmn/J mice allowed a longer bedding replacement interval.

PC123

Drugs Used for Sedation Influences Mosquito Feeding and Viability in Malaria Transmission

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Abstract
To infect *Anopheles stephensi* mosquitoes with malaria parasites (*plasmodium cynomolgi*), the mosquitos are offered parasite-infected blood. The malaria infected blood comes from experimentally infected resus macaques (*Macaca mulatta*). After *blood* withdrawal the infected blood is placed into a glass holder covered with a parafilm membrane through which the blood can be ingested by the *mosquitoes* by piercing the parafilm. The blood withdrawal from the infected monkey takes place ∼14 days after the infection of the monkey and is performed while the monkeys are sedated. We have been performing these types of experiments since 2009. In 2019, the sedation protocol was refined from ketamine alone to a combination of ketamine and medetomidine (α2 agonist) to enhance the sedation and analgesia. This combination is known to provide better analgesia, deeper anesthesia, and a shorter recovery. In addition, the use of medetomidine accommodates a decreased dose of ketamine, thus reducing muscle necrosis and inflammation caused by a high ketamine dose.

We observed that the mosquitoes were behaving differently during and after the blood-feeding when the enhanced sedation protocol was applied. The mosquitoes did not disengage after engorging and would drink until they fell from the feeder (on top of the cage) onto the bottom of the cage. This resulted in increased mosquito mortality.

After reversion of the sedation protocol from the combination ketamine-medetomidine back to ketamine alone, the mosquitoes showed their 'normal' feeding behaviour again and the mortality rate decreased.

These observations suggest a role for medetomidine in mosquito feeding behaviour and mortality.

PC124

Minimal Invasive Contraceptive Implants in Female Macaques

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Abstract
Contraception is often necessary for managing captive group-housed non-human primates. Etonogestrel (ETO) implants are subdermal, progestin-only contraceptives that are reversible and long-acting for at least three years, used in humans as Implanon® or Nexplanon®.

A retrospective data analysis was conducted to evaluate the contraceptive effectiveness and reversibility of subdermal placement of 1/4 or 1/3 of an ETO implant (68 mg/implant) in 129 female rhesus macaques (Macaca mulatta) and 67 cynomolgus macaques (Macaca fascicularis) at the Biomedical Primate Research Center in Rijswijk, Netherlands. Additionally, single cross-sectional ETO serum concentrations were measured, and hemoglobin and blood chemistry were evaluated prior to ETO insertion and at three in-treatment timepoints. Ultrasound data were also collected to assess the influence of ETO on uterine volume and endometrial thickness.

We observed an effectiveness of 99.80% and 99.95% with ETO lasting up to three years in rhesus and cynomolgus macaques, respectively. Median ETO durations of 1.2 years (range 0.1–6 years) and 1.9 years (range 0.6–4.7 years) showed median serum concentrations of 112 pg/mL (range 0–305 pg/mL) and 310 pg/mL (range 183–382 pg/mL) for rhesus and cynomolgus macaques, respectively. Extended ETO durations in 22 macaques (range 3.1 – 5.0 years) resulted in no unintended pregnancies. After removal, 17 females were allowed to breed again, resulting in 82% uneventful deliveries. In addition, ETO had no clinical effect on hemoglobin and blood chemistry parameters nor on the uterus.

This study indicates that both 1/4 and 1/3 of an ETO implant are effective, long-acting, reversible, and safe contraceptives for use in macaques.

PC125

Managing of Barbering in an Animal Facility as a Benefit for 4R

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Abstract
Aim: For a long time, barbering was underestimated as a relevant topic regarding animal welfare in animal facilities. In Germany, there is an ongoing discussion about how to deal with animals that show barbering and about the impact of barbering on experimental results. Due to the lack of recommendations on how to deal with barbering, we have established a standard step-by-step procedure for the different occupational groups in our animal facility as a strategy due to the 4R principle.
Methods: To manage barbering, we have focused on the main questions and challenges animal technicians, scientists and responsible veterinarians are dealing with in their daily working routine in animal facilities. Our approach includes intensive educational programs, thorough documentation to identify related factors influencing barbering, a clearly defined cascade of action and an improved way of communication.
Conclusion: Since the etiology of barbering is not fully understood and recommendations are missing, we have developed an inhouse step-by-step procedure to address that challenges in our

animal facilities. This procedure focuses on understanding the mechanisms of barbering, identifying various influencing factors, assessing the burden on both - barbers and barbered animals - and exploring potential strategies to prevent barbering in animal facilities.

PC126

Improving Camelid Welfare Using a Mobile Application, Data Visualization and Analytics Software

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Abstract
This abstract introduces an innovative animal health monitoring application specifically designed for camelids used in biomedical research and development. The conventional process of monitoring animal health has been associated with substantial data generation. Formerly reliant on manual record-keeping within stable environments, the workflow involved transcription into notebooks and Excel-based tables, resulting in extensive administrative burdens for animal caretakers and veterinarians. This approach also posed challenges in sharing data among multiple veterinarians and tracking long-term issues. Moreover, there was a need for enhanced data visualization and interconnectivity.

Addressing the lack of suitable health monitoring tools for large research animals, the Camelid Health Check application (CHC) was developed in-house. This mobile application allows offline data recording using a tablet in the field and facilitates the creation of real-time health monitoring reports. Upon synchronization, these reports are integrated into a centralized database and made accessible to the multidisciplinary team through a web-based application. Furthermore, a data visualization tool allows the linkage and visualization of data, enabling comprehensive analysis of large datasets and the generation of insightful monitoring reports.

This application significantly streamlines the administrative processes, enhances data quality and accessibility, and facilitates collaborative decision-making among veterinary professionals which results in improved animal care and welfare standards.

PC127

Optimizing Procedures for GLP Study of Minimally Invasive Vertebroplasty in Experimental Sheep

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Abstract
Sheep are an appropriate experimental model for evaluating the safety and efficacy of a novel bone cement vacuum-guided device during the preclinical stage. However, the vertebral size of sheep is only half that of humans, and sheep lack osteoporosis, posing a significant challenge for clinical orthopedic surgeons to perform minimally invasive vertebroplasty successfully. A preliminary training phase using sheep cadavers for human surgeons to refine their techniques under real-time C-arm fluoroscopy and identify optimal angles of bone needle insertion. Consequently, vertebroplasty procedures were successfully completed in eight live sheep without complications. Postoperative observations confirmed normal mobility and the absence of adverse events. By enhancing procedural precision through cadaver practice, the need for repeated live animal trials was minimized, thereby promoting animal welfare and aligning with the principles of the 3Rs (Replacement, Reduction, Refinement). Moreover, the results of this study facilitated the approval of the bone cement vacuum-guided device under the U.S. FDA's STeP program, enabling accelerated regulatory support for market entry while demonstrating a commitment to ethical preclinical practices.

PC128

Evaluation of Structural Environment and Implementation of Novel Foraging Strategies in NHP/Large Animals

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Abstract
Enrichment may support positive psychological well-being and encourage natural behaviors in captive animals. Foraging devices promote a species-typical activity that dominates the time budget of primates outside captivity and provide inherent cognitive challenges, physical activity demands and multisensory stimulation. The newly developed foraging device "feedme" has been tested in a pilot study and further observations of tests in various facilities will be presented.

The aim of the pilot study was to test the foraging device to determine its effectiveness in increasing foraging behavior and to determine its longevity in cynomolgus macaque breeding groups. Further observations have shown that several parameters like positioning, number of animals, number of devices, etc. have a considerable influence on the interaction with the device. Secondly, possible improvements to the device should be identified that would enable higher efficacy and thus more efficient enrichment.

The position of the device must be well identified. Depending on the group size, more than one device may be required. The interaction times of males, females and juveniles with the device varied. Juvenils spend the longest time. This device can circumvent food aggression, although in females did display some food aggression during behavior scans. To sum up, the device has a positive influence on animal well-being, but there are further studies ongoing.

Furthermore, several studies are running in other large animals like horses, dogs and pigs. Preliminary results are showing positive effects on animals health care.

PC129

Aging, not Illness. *Veterinary Care for Old Laboratory Animals in Research*

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Abstract
The use of aged animals in biomedical research provides invaluable insights into the biological processes of aging and age-related diseases. However, distinguishing the phenotype of an aged experimental animal from that of a sick animal remains a critical challenge. At our institute, dedicated to aging research, we address this issue through comprehensive veterinary support and phenotypic characterization of aged animals.

This poster focuses on the specific needs and phenotypic traits of aged killifish (e.g. Nothobranchius furzeri) and mice, two key model organisms in aging research. For killifish, particular attention is given to their increasing relevance in gerontology studies and the challenges in differentiating normal age-related changes from pathological conditions. For mice, we illustrate key differences between young and aged individuals, highlighting age-associated physiological and pathological changes.

We will present a systematic approach to identify and document age-related phenotypes, supported by photographic comparisons of aged versus diseased individuals in both species. The phenotypes of aged animals, including both fish and mice, are integrated into our scoring sheets to ensure accurate monitoring and evaluation.

By understanding the unique characteristics and requirements of aged experimental animals, researchers can improve data quality, enhance animal welfare, and better interpret findings in the context of aging. This poster aims to provide practical guidance for the research community, fostering a deeper appreciation of the complexity of aging in laboratory animal models.

PC130

Assessing Lidocaine-based Analgesia for Mouse Ear Notching: Insights into Strain-Specific Reactions

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Abstract
From the beginning of 2024, a new national law on animal protection in Finland (1165/2023 3 §) requires laboratory rodent ear notching to be performed only with "appropriate pain relief". However, the European Union directive on the protection of animals used for scientific purposes (2010/63/EU) classifies ear notching as a routine identification method rather than a procedure, leaving no specific guidelines on suitable analgesia at the EU or the national level. This study aimed to evaluate the suitability of a lidocaine-based cream as analgesia (EMLA cream 5%, lidocaine + prilocaine 25 + 25 mg/g, Aspen Pharmacare Australia) for mice undergoing ear notching.

The experiment involved 15–33-week-old male and female C57BL/6JRj mice and 17-week-old male BALB/cJRj mice. EMLA was applied 10, 15, 20, or 30 minutes before ear notching. Reactions to notching were observed and categorised into three levels: no reaction (0), mild (flinch, 1) or marked reaction (head shake/vocalisation, 2).

Most C57BL/6JRj mice displayed marked reactions at 10–20 minutes post-application (e.g., 20 min: 83% marked, 8% mild, 17% no reaction, n = 13). In mice that were marked with 30 minutes post-application, reactions were significantly reduced (27% marked, 13% mild, and 60% no reaction, n = 30). In contrast, BALB/cJRj mice showed minimal responses even at 10 minutes (70% no reaction, 30% mild, n = 10).

Preliminary results suggest EMLA is effective if applied 30 minutes before ear notching, but strain-specific behavioural differences must be considered, as BALB/cJRj mice exhibited lower sensitivity. Further research is required to evaluate pain caused by ear notching more comprehensively.

PC131

Buprenorphine to Alleviate Pain in Pigs Undergoing Experimental Surgery – A Systematic Scoping Review

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Abstract
Background: Pain is unavoidable in experimental surgical animal models and must always be assessed and alleviated to improve animal welfare and comply with the 3Rs. Buprenorphine has been used as a postoperative analgesic in experimental animal research for decades. The pain modulating effect of buprenorphine is long compared to most opioids and it was the most frequently used postoperative analgesic drug in porcine animal models between 2012–2014 (1). Recommended dosages to pigs are based on knowledge from various other species than the pig. Detailed information about the drug in terms of pharmacokinetic properties, adverse effects, therapeutic interval and whether a ceiling effect is observed specifically in the pig may be limited. The aim of this species-specific project was to investigate published knowledge concerning buprenorphine use in pigs.
Methods: A systematic scoping review methodology was chosen because of the broad nature of the research question. The aim was divided into several subcategories to be able to perform an evidence-based search. It was decided to include both publications and grey literature in the search based on more than one search string. A detailed protocol was developed to maintain a transparent and thorough investigation of each research question.
Results: The preliminary search strings are developed, and the final results are on the results it is possible to improve pain-alleviation in pigs even further and disclose areas of missing information.

PC132

Setting a New Standard for Animal Welfare in the Animal Provider and Services Industry

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Abstract
As a leading provider of rodent models, Taconic is committed to upholding animal welfare best practices for our animals while offering the best solutions to our customers. The AWARE™ (Animal Welfare Aligning with Resource Efficiency) Program demonstrates our dedication to drive awareness of animal welfare.

The AWARE™ Program empowers our staff and researchers in the scientific community to proactively plan, execute, and monitor breeding projects. With the AWARE™ Program, we ensure projects are designed to fulfill the scientific needs of the research project while maintaining a keen eye on animal welfare. The launch of this program is a key step in creating an industry-leading framework guiding the monitoring of animal welfare observations, animal utilization, and adverse phenotypes.

The AWARE™ Program consists of two main pillars that are closely related:

The Design & Planning arm considers best practices, preventive measures, and the 3Rs in every colony managed at Taconic. The goal is to ensure animal model production levels are closely regulated based on demand.

The Monitoring & Reporting arm, where we focus on defining appropriate performance indicators, monitoring thresholds, and animal utilization metrics. This is done to maximize resource efficiency and reduce the number of animals needed.

The overall goals of the program are:

- Reducing animal wastage while guiding researchers to more robust study design
- Increasing awareness and transparency of potential pain and distress for the animals and proposing measures to reduce this
- Foster a culture of care and reduce potential compassion fatigue

PC133

Successfully Implementing the CoP Perioperative Care by Hands-on Training in a Dry Lab

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Abstract
In 2018 a group of field experts in the Netherlands set up a working group which created a Code of Practice "perioperative care" (published in 2022). This CoP is a practical document for researchers and biotechnicians who need to perform surgery on experimental animals. The different chapters are divided in a pre-operative, intra-operative and postoperative phase and lead you step by step through the surgical process.

To ensure that these guidelines were put into practice, a special skills lab was established in the animal facility of the Radboud UMC Nijmegen. Every researcher and biotechnician that wants to perform a procedure on a lab animal must be proven competent. To acquire this competency, they can register to be trained in a variety of procedures. When researchers or biotechnicians need to perform surgery, they are obliged to follow the dry-lab for "peri-operative care" before they start with surgeries on alive animals. Training is given by designated highly skilled senior biotechnicians in a designated surgery room equipped for this purpose. People that register for this dry-lab prepare themselves by reading the Code-of-Practice Perioperative Care prior to the training. During the skills lab training, the trainer discusses the theoretical part of how to reach optimal peri-operative care by using a PowerPoint presentation. This part is followed by a hands-on training on practical issues, like how to create and maintain an aseptic environment and how to monitor and report physiological data. This will all contribute to improved care and welfare, resulting in refinement and reduction.

PC134

Together or Alone? Huddling Energetic Savings in Three Social Mole-rat Species

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Abstract
African mole-rats (Bathyergidae) are strictly subterranean rodents distributed in sub-Saharan Africa. Although soil layer provides a temperature buffer, the temperature in their burrows is usually below their thermoneutral zone and thermogenesis is necessary to maintain stable body temperature. In social bathyergids, an important mechanism for decreasing the thermoregulatory cost is social thermoregulation - huddling. An effect of huddling may be of special importance especially during the forming of asocial groups when usually only two adults are present, because of higher heat loss from bodies of social species compared to solitary mole-rats.

In our study, we measured resting metabolic rate and energetic saving in three social bathyergid species differing in body size in animals housed in pairs as well as housed individually at two different ambient temperatures (T_a). At a temperature within their TNZ ($T_a = 30°C$), no energetic savings were expected, whereas in $T_a = 20°C$ we expected energetic savings due to huddling.

We found no energetic savings at 30°C in either species, but almost 20% in two small species *F. micklemi* and *F. anselli* at 20°C. In the largest species, *F. mechowii*, no significant energetic savings were observed. Our results confirm the importance of huddling for energetic balance of social mole-rats and show that huddling with one partner can bring remarkable energetic savings, which can be allocated to other purposes such as extension of established burrow system or reproduction to increase workforce and fulfill the purpose of dispersal.

PC135

Effect of Acclimatization and Housing Temperature on Metabolic Activity in Mice

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Abstract
Mice need to be acclimatized to a new housing condition before starting measurements or experiments to avoid unwanted effects of stress. However, there is no consensus on how long it takes to achieve adequate acclimatization. This is because acclimatization depends on the facility, the experiment itself, the previous events/procedures, animal handling, and many other factors. We have studied the acclimatization process in our indirect calorimetry system to obtain constant metabolic readings. As the total daily energy expenditure is highly dependent on the ambient temperature, we investigated whether changing the housing temperature to thermoneutrality (30C) compared to the standard housing temperature (23C) influences the acclimatization in male and female C57BL6/J and BALB/c mice (15–16 weeks old). Mice were continuously housed in the indirect calorimetry system for 14 days. For the first 7 days the mice were housed at either 23C or 30C. Thereafter, the temperature was changed to 30C or 23C respectively. After 14 days, the mice were sacrificed for post-mortem analysis of adipose tissue *UCP1* and plasma corticosterone levels. Our preliminary results suggest that our animals adapted to a temperature change from 23C to 30C and vice versa within one day. In addition, we observed that mice acclimatized to the new environment in less than two days when first placed in the indirect calorimetry system. Based on our data, we show that a short acclimatization period is sufficient, allowing for a higher throughput or more sub-experiments within a given time frame, without compromising the welfare of the mice involved.

PC137

Raising Bearded Dragons: Advances in Husbandry, Health and Breeding

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Abstract
Bearded dragons (*Pogona vitticeps*) have become an established animal model for research on neuronal circuits, behavior and sleep at the Max Planck Institute for Brain Research. They show a wide range of complex and often visually driven behaviors.

Standardized husbandry and breeding procedures were continuously developed and optimized over the past years. This enables us to now breed all animals in-house and in accordance with scientific demand.

The animals are regularly housed individually in standardized and enriched terraria, with a defined feeding schedule and carefully controlled lighting conditions. A strict hygiene concept and health monitoring successfully eliminated adenovirus, a known pathogen, from our colony. Routine parasitological examinations, regular weight and length monitoring, and targeted treatments ensure a clinically healthy population. Animals are bred after hibernation, and ultrasound monitoring helps to reach the required number of hatchlings.

Backed by careful and complex planning, our successful breeding and rearing program ensures a reliable, continuous supply of healthy, suitable animals for research. A rearing success rate around 99% highlights the effectiveness of standardized husbandry practices and a commitment to animal welfare.

PC138

Challenges Associated with Female Rabbits Group Housing in Local Tolerance Studies for Vaccines

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Abstract
Social housing and allowing expression of natural behaviors are key components of animal welfare. To this end, we designed custom-made pens to enable female rabbits group housing in local tolerance preliminary studies for vaccines.

One 7200 cm2 pen can house up to 3 rabbits of less than 5 kg and two pens can be joined to house up to 5 rabbits. Animals are housed on wood shaving bedding with following enrichment items: hiding tunnel, hay logs to eat, wooden stick to gnaw and stainless steel toys to manipulate. Observation revealed high animal welfare benefits such as increased activity compared to individual cages housing and expression of digging, standing, stretching and resting in close contact behaviors. However, skin abrasions were sometimes detected on the back at the beginning of the acclimation period and attributed to mounting. Despite these lesions being transient and self – recovering, the acclimation was increased to 15 days to avoid interference with assessment of local tolerance of the tested vaccines. A slight increase of body temperature was noted compared to historical data on individually housed rabbits but this change was not considered as disqualifying. On few occasions, more severe skin lesions were observed and led us to reinforce the enrichment program. Close monitoring by staff is also mandatory to have an early detection of conflict and take appropriate actions such as increasing surface areas, adding enrichment or separating incompatible animals. Social housing in rabbits can be challenging, requiring behavioural management and programmatic flexibility to be successful.

PC139

A Project Based Approach: Refinement of Animal Welfare in Tumor Models

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Abstract
A substantial amount of the animal experiments within our institute uses tumor models. Like in human disease tumors affect welfare in mice in different ways. Depending on the model this can, for example, include weight loss, bad fur condition, irritation of the skin, ulceration or restricted blood flow. Not all welfare aspects are observed in all tumor models and therefore model-specific welfare monitoring is required. Therefore, tailor made monitoring requirements and humane endpoints are needed.

Approach: A project was initiated to facilitate researchers during the design phase of their experiment a document is being made that provides relevant information about various aspects of their tumor model, welfare monitoring, refinement aspects of the experiment and accompanying humane intervention points (HIPs) and humane endpoints (HEPs). Such a guiding document is useful as it establishes awareness about welfare aspects that are linked with a specific tumor model. Also, the Animal Welfare Body (AWB) can consult this document when advising on model specific welfare issues or refinement aspects when advising on projects or working protocols. To support the monitoring of animal welfare and the execution of HEPs in the execution phase of these experiments well-defined scoring lists were created by our biotechnicians, animal caretakers and researchers. These scoring lists include photos and a description of the stages of tumor development in several mice strains. Using this scoring list contributes to refinement by creating consistency in monitoring of welfare and in applying HIPs and HEPs.

PC140

Zebrafish Environmental Enrichment: A Behavioural Assessment

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Abstract
Enrichment is widely accepted to be essential to the welfare of laboratory animals, and in other species such as mice and rats is a standard practice in housing across most facilities. Many zebrafish facilities however house fish in barren tanks with no environmental enrichment. A trial was conducted to assess the effects of environmental enrichment on zebrafish behaviour using a novel tank test. Overhanging plastic grass in the home tank was used as a simple form of enrichment, attempting to resemble the kind of plant life zebrafish would have in their natural river environment. Other potential impacts of the enrichment were assessed, such as changes to water flow and in tank breeding success rates. The results suggest this enrichment is a refinement in the housing of zebrafish and encourages the expression normal behaviour.

PC141

Improved Welfare Monitoring Via Continuous Activity Recording in Home Cage Connected to Machine Learning

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Abstract
In every facility, daily controls by animal caretakers are performed to ensure animal welfare and well-being. Access to food and water, identification of clinical signs of distress or pain and humane experimental endpoints are usually checked during this control. These visual observations represent a snapshot during the inactive period of rodents and during a short period of time (minutes) per cage. The presence of enrichment material (tunnels,

nesting material, etc.) reduces the capability to carefully view all the animals without opening the cage. To improve these controls, we investigated the benefit of the use of digital home cages equipped with sensor technologies that continuously track the movement of animals. We hypothesized that differences in home cage activity patterns would predict impairment of animal welfare and help the animal facility staff to identify clinical cases. The activity pattern of 229 observed clinical cases with our standard procedures representing 70 different mouse strains housed in digital cages from May 2020 to February 2024 were retrospectively analyzed using Machine Learning (ML) models to generate digital alerts and identify potential clinical cases. Our results show that the ML algorithm generates digital alerts in 84% of the observed cases within 7 days before the clinical signs were reported by the animal caretakers. These data suggest that increasing monitoring with AI algorithm could help improving animal welfare control by assisting the animal caretakers in their daily observations.

PC142

Refinement by Gentle Handling of Mice Affects Oral-dosing Pharmacokinetic End Points and Reduces Stress

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Abstract
Lifting mice by their tails is a common handling method used for laboratory mice, yet it causes substantial stress. Alternative handling methods have a positive impact on animal welfare, but, there are limited studies on the effects of handling and habituation on scientific endpoints; hindering implementation and refinement in academia and industry. This study aimed to examine the impact of different handling approaches (tail lifting vs. tube lifting) and habituation to handling on drug absorption, exposure, and welfare indicators in a 24-hour pharmacokinetic study, conducted in an industry setting. CD1 mice were subjected to three conditions: tail lifting without habituation, tube lifting without habituation, or tube lifting with a 10-day habituation protocol. Following oral gavage administration of mexiletine, a 24-hour pharmacokinetic study was conducted . Results showed that the habituated group exhibited a higher maximum serum concentration (Cmax), shorter time to Cmax (Tmax), and 30% greater drug exposure compared to the tail and tube-lifted groups. These findings correlated with reduced stress levels, as evidenced by lower facial grimace scores in the tube-lifted groups relative to the tail-lifted group. After repeated blood sampling, the habituated group demonstrated the highest level of handler interaction, with only this group voluntarily approaching the handler. The findings suggest that stress induced by tail lifting, oral gavage, and blood sampling leads to decreased drug uptake and exposure. This stress can be mitigated through gentle handling and habituation, potentially resulting in more accurate pharmacokinetic data, enhanced scientific quality, and improved animal welfare.

PC143

Aggression in Male Laboratory Mice – an Epidemiological Approach

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Abstract
Mice are social animals and group housing is fundamental to ensure good animal welfare. Group housing is also required by the Directive 2010/63/EU. However, aggression between male mice is a major challenge in laboratory mouse husbandry, affecting both animal welfare and scientific outcomes. Identifying key factors associated with aggression is thus crucial to refine present husbandry routines and mitigate or prevent this problem.

In a previous study done in the US (Theil et al 2020), we used an epidemiological approach to study mouse aggression, a powerful and non-invasive method to address important welfare concerns and increase the understanding of environmental and biological factors affecting multifactorial problems. In the present study, we applied the same protocol to collect data from several Swedish mouse facilities. Rooms and racks were randomly selected, and cages visually assessed to collect data on fighting and factors potentially influencing home-cage aggression.

A total of 4614 cages were observed during one year, 1638 of which contained group housed male mice (4933 males in total). Males were observed fighting in 19% of the cages. Further results on factors associated with aggression, including the translation from US to European husbandry norms, will be presented. Notably, almost 10% of the mice were single-housed, with time spent single ranging from days up to one year. According to the Directive, when being singly housed, the duration shall be limited to the minimum period necessary. The question of how "a limited period" can be defined will therefore also be discussed.

PC144

Habituating Mice to Metabolic Cages Prior to Experimentation to Reduce Acute Stress

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Abstract
To understand the value of handling and habituation of rodents used in scientific studies, and to assure accurate data from animal studies, there is a need to assess whether mice and rats habituate to metabolic cage housing. Animals are single housed in cages with mesh wire and without bedding and enrichment. Such cages are used to collect urine and faeces for investigation of metabolic conditions such as kidney function. It is known from previous studies that metabolic cageing transiently but significantly affects rodent body weight.

Corticosterone is the primary stress-responsive glucocorticoid synthesized by rodent adrenal glands, and acute stress results in increased excretion of their metabolites in urine and faeces.

The study consisted of placing 14 male mice in metabolic cages three times: baseline (met cage 1), after two weeks (met cage 2) and after another two weeks (met cage 3). Using enzyme-linked immunosorbent assay (ELISA), corticosterone levels from urine samples from all three metabolic cage samplings were compared.

The data shows that mice have significantly less corticosterone levels on second and third events of metabolic cage housing. On the second occasion, corticosterone levels were reduced by more than 50% compared to the first occasion. Between the second and third occasion there was no significant decrease, suggesting stress levels due to metabolic cageing may be more stable after only one metabolic cage habituation.

Besides habituation, handling during cage changes until metabolic cage occasions 2 and 3 could also have an effect on the reduced corticosterone levels.

PC145

Refining Weight Monitoring in Rhesus Macaques (*Macaca mulatta*): Use of Percentile Growth Curves

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Abstract
Weight loss is a key metric in animal health and welfare. In laboratory research certain levels of weight loss (15% and 20%) are commonly used as humane endpoints. However weight loss is usually a calculation that relies on the most recent recorded weight and fails to capture whether an animal is growing as expected. Juvenile and adolescent animals should still be growing and increasing in weight. In human health, percentile growth curves for weight and other measurements are used to identify whether a child is growing as expected. We propose that percentile growth curves are a more refined way of monitoring growth in rhesus macaques and take into account healthy growth.

We used 15 years of breeding colony weight records to construct percentile growth curves for male and female rhesus macaques (Macaca mulatta; 8291 weights in total from 830 macaques; taken between 2008 and 2023). We used the GAMLSS package in R to fit Lamda-Mu-Sigma models to the weights (separate models for males and females). This model allows both the plotting of an individual animal's weight across time on the centile growth curves and given an animal's weight and age to calculate a Z-score.

We demonstrate with case studies how percentiles and Z-scores can be used to capture both weight loss and failure to grow and how events such as procedures and injuries may impact growth. This is now being used on a routine basis in the macaque breeding colony to identify animals with potential weight issues.

PC146

Eradicating Sentinel Dependency: Animal Free Enhanced Detection in Rodent Health Monitoring

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Abstract
Fera has launched an innovative system for detecting pathogens in research animals in-line with the 3R's.

In 2023 Fera's Animal Health Monitoring Team collaborated with Aberdeen University to identify a robust method of monitoring that would remove the requirement for sentinel animals whilst maintaining the quality of results obtained.

Fera's animal health team identified collection media which could be placed in a cage and could act as a sentinel. The team also identified a method to overcome nucleic acid degradation and environmental contamination which is often a concern in environmental screening.

Aberdeen University initially trialed the new screening method versus sentinel animal screening and the results demonstrated that this method of screening provided a more accurate representation of rodent colony status.

Multiple facility trials carried out since the initial Aberdeen trials have demonstrated that the Ghost Sentinel® is unparalleled in terms of its detection rate compared to other methods of screening for the agents that are prevalent in research animal colonies, removing the requirement for animals in routine health monitoring.

This poster will describe the validation of the animal free Ghost Sentinel®, the method of application and the results obtained versus current screening strategies.

PC147

Genetic Quality Control: Essential Tools for Research Reproducibility

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Abstract
The expanding utilization of genome editing technologies and increasingly complex genetically modified mouse models necessitates robust genetic quality control measures to ensure research reproducibility. This presentation addresses three fundamental aspects of modern genetic quality control in laboratory animal research. First, the ICLAS Laboratory Animal Quality Network's GENRef program serves as a vital resource by providing validated reference DNA from common inbred strains. This enables laboratories worldwide to perform accurate genetic monitoring of their colonies and validate genetic backgrounds of research models.

Second, comprehensive genetic quality management encompasses multiple critical components: implementation of careful breeding strategies, maintenance of detailed colony records, systematic validation of genetic modifications, and regular monitoring of genetic background integrity. These practices are essential for maintaining the genetic integrity of research animals and ensuring experimental reliability. Finally, the newly established Laboratory Animal Genetic Reporting (LAG-R) guidelines introduce a standardized framework for documenting genetic information in scientific publications. This framework addresses the current challenges in genetic reporting consistency and promotes transparency in animal model documentation. The integration of these tools, practices, and guidelines creates a robust system for maintaining and verifying genetic quality in laboratory animals, thereby enhancing research reproducibility and scientific rigor in biomedical research. This comprehensive approach to genetic quality control is becoming increasingly crucial as the complexity of genetic modifications and experimental designs continues to advance.

PC148

Oral Self-administration of Piritramide in C57BL/6JRj Mice Following Laparotomy: Cage-side Pain Assessment Evaluation

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Abstract
Effective post-operative pain relief is crucial for the welfare of laboratory mice. Piritramide, an opioid used in human medicine1, has been proposed as a potential analgesic for laboratory animals. This study assessed its pain relief effectiveness when given through drinking water (d. w.) by monitoring physiological and behavioral markers of pain at baseline and after laparotomy.

Baseline data on water consumption, food intake, body weight, wheel running, and nesting activity were recorded for group-housed male and female C57BL/6JRj mice ($n = 10$ per sex) over 4 days and then reassessed after surgery with daily clinical evaluations. A laparotomy to inject tumor cells into the liver lobe was performed under isoflurane anesthesia, with buprenorphine (0.06 mg/kg) given subcutaneously pre-surgery. Post-operatively, piritramide (0.068 mg/ml) was administered via d. w. for 4 days.

D. w. intake in female mice showed no significant differences compared to baseline, while male mice had a significant reduction 4–7 hours after surgery ($p = 0.0229$). Dry food intake remained below baseline for all cages, but higher amounts of wet food consumed offset this. Wheel running parameters remained stable also after surgery, and nesting behavior showed no significant changes in both sexes. There were no body weight losses greater than 4.5%, and clinical scoring showed a mild score of 2 in 14/100 observations.

The results indicate that a single injection of buprenorphine, followed by self-administration of piritramide, effectively manages pain in mice undergoing moderate surgical procedures. More research is needed to confirm piritramide's analgesic effectiveness and oral pharmacokinetics.

PD001

Mental Health of Apprentices During First-ever Experience with Laboratory Animals

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Abstract
During their initial exposure to laboratory animals, apprentices' mental health is a crucial factor in fostering a sustainable and empathetic approach to animal care and research. This study focuses on first-year apprentices, predominantly under 20 years old, who encounter laboratory animals within the first three months of their training. During this period, they participate in a tailored animal-handling course equivalent to FELASA/LTK-1 standards. The course emphasizes a gradual introduction to animal handling, conducted in small groups with close supervision. Dedicated spaces for reflection and access to supportive conversations are provided to address emotional challenges.

To assess the mental health impact of this first contact, we conducted an anonymous survey using a ranking-based questionnaire. The survey aimed to evaluate the apprentices' mental state post-first exposure and identify specific methods or tasks perceived as distressing. By analyzing these data, we seek to ensure a strong mental foundation for apprentices' future work with laboratory animals.

Our approach at Roche emphasizes the importance of gradual, low-pressure introductions and refinement techniques (e.g., gentle acclimatization methods such as rat tickling and practice with plastic probes) in reducing stress. These measures contribute to a more humane and psychologically supportive training environment.

PD002

Scoring Housings for Macaques: A Tool to Foster Husbandry Improvements

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Abstract
Housing enclosures and husbandry conditions for Non-Human Primates used or intended to be used for scientific purposes are extremely diverse between institutions and even intra-institution. The 2010/63/EU Directive defines minimum standards required for

the care and accommodation of each species, but a good culture of care would foster to go beyond minimal requirements. In this context, we developed an innovative scoring system for macaque housing based on different principles known to contribute to good welfare of animals such as used in the Welfare Quality® project[1] or the five domain model[2]. Housings are scored according to four principles (Good feeding; Good housing; Appropriate behaviour; Animal management) divided into 12 criteria and 35 indicators. Indicators are measures of the animal's environment such as structural elements and environmental parameters, opportunities to perform various behaviours, as well as human-animal interaction practices. This scoring system finally allows to classify each housing as Basic, Satisfying or Optimal. We assessed the tool within SILABE Platform of University of Strasbourg, France, for different housing enclosures before and after renovation, identifying key elements to go beyond basic housing. This approach can be implemented in various institutions to help identifying construction and renovation needs, enrichment programs and husbandry practices that could be improved to reach Optimal housing conditions. This tool can be extended to other species with different graduation adapted to specific needs and therefore benefit to all animals under human care.

PD003

Leadership Strategies for Cultivating a Culture of Care in a Laboratory Animal Facility.

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Abstract
Fostering a culture of care for animals and animal care staff are equally important, since one cannot thrive without the other. The Culture of Care at Novo Nordisk starts with our staff who must provide the necessary care to the animals with an empathic approach. The purpose is to create a healthy, caring culture which enables us to achieve our goals in relation to optimizing the animal welfare and the wellbeing of the people working with them. This requires general support from management to prioritize animal welfare, both during normal husbandry and on specific projects, ensuring a psychologically safe environment where you are not afraid of making mistakes. It is important to realize that a culture is not build or sustained by itself. In an animal facility, different work categories meet, technical personal, veterinarians, academic personal with different backgrounds and cultures. Building a caring culture requires intentional effort and focus on fostering empathy, respect and inclusivity. It also requires an organizational structure that support achievements within animal welfare in its own value. This presentation will give insights into this work, and give tangible, day-to-day practices, examples and take-home ideas on how we actively work with fostering a culture of care.

PD004

Applying Six Sigma to Strengthen the Culture of Care

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Abstract
To ensure high staff motivation in laboratory animal science, more than just good intentions are needed – a solid operational foundation supporting effective staff planning and workload management is crucial. The challenge is amplified by working closely with scientists, who assign important tasks and provide input that directly affects workload. Using the Six Sigma Lean methodology, we demonstrate how to transform HR management into a data-driven process while fostering a stronger culture of care and motivation.

Our approach starts by identifying workload peaks through data collection and visualization techniques. We gather historical data on weekly task volumes, categorize them, and consider non-measurable activities like communication. The results revealed uneven task distribution, with significant workload spikes after vacation periods. We then incorporated data on employee vacation and sick leave to explore possible links between high workloads and absenteeism. At the same time, we analyzed motivation levels to understand their relationship with workload fluctuations.

Our findings show that implementing an annual planning framework based on Six Sigma principles can significantly improve both vacation and staff planning. By addressing workload peaks early and reallocating tasks strategically, companies can achieve a more balanced workload, boost employee motivation, and maintain sustainable peak performance. This approach not only improves daily operations but also fosters a healthier, more resilient work environment for all team members.

PD005

Scenarios Used as a Basis for Discussing Culture of Care in Animal Research Facilities

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Abstract
Workplace culture evolves continuously through the interplay of supporting structures, individual experiences, and personal mindsets. It shapes how we communicate, collaborate, and address challenges. In animal research, this culture is evident in how we discuss and handle animals, reflecting shared values and practices. Proactively creating a process for Culture of Care discussions in a neutral, structured setting is essential for the fostering of intentional improvement of the workplace culture. It ensures that the dialogue can remain constructive and forward-thinking,

avoiding a focus on past mistakes or grievances. This approach encourages open and honest participation from all co-workers, promoting trust and collective growth.

To support proactive and forward-thinking dialogues, the Swedish 3Rs Center has developed a resource containing 25 fictive scenarios based on stories from animal research facilities, paired with reflective questions. These scenarios serve as a practical tool to initiate open, neutral discussions among co-workers, promoting mutual understanding and consensus on handling complex situations. Regularly engaging with these scenarios as part of routine activities can help foster positive workplace dialogues. It can also promote a shared commitment to Culture of Care while developing a thoughtful, supportive environment that enhances continuous improvement of both human and animal welfare.

PD006

The 3Rs Champions Team: Empowering Animal Technicians

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Abstract
Animal Technicians are the powerhouses of animal facilities and, to reflect their knowledge and skills, we have created a 3R's Champions team to develop, promote and deliver 3R's improvements.

Core members include technicians from breeding and experimental facilities, a rederivation technician, the experimental facility NACWO and the NIO (the University's NC3Rs 3Rs Champion). Regular meetings allow open discussion within the group where all suggestions for 3R's improvements are reviewed and, in consultation with the NVS and Director of Biological Services, a decision is made on next steps.

The function and outcomes of the 3R's Champion's group are communicated to the research community and the AWERB via a variety of forums. This thereby encourages researchers to approach technicians with improvements to individual studies and to be co-opted to the 3Rs champion team. This has opened a communication channel for researchers to acknowledge and appreciate the experience and skills of our animal technicians.

Creation of a technician driven 3R's Champions team can promote empowerment and confidence to communicate across groups within a research environment to implement the 3R's, improve welfare and enable better scientific outcomes.

PD007

Empowering Animal Welfare Bodies at the Epicenter of the Culture of Care strategy

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Abstract
Animal Welfare Bodies (AWB) play a pivotal role in the culture of care strategy of research institutions. They ensure that the ethical treatment of animals is not only a regulatory obligation but also a core principle of research practices. To cultivate this culture effectively, AWB must possess sufficient authority and resources, including access to training materials, funding for welfare initiatives, and the ability to influence institutional policies.

This presentation will illustrate, based on case studies, how an AWB with the necessary authority can implement rules and recommendations that go beyond basic compliance, adapting them to the specific cultural and contextual needs of the institution. This flexibility is essential, as various research environments may face distinct challenges and opportunities in animal care.

Furthermore, we will demonstrate how AWB can significantly influence the content and delivery of training programs, thereby enhancing long-lasting competencies and philosophies of animal caretakers, which is crucial for fostering a comprehensive understanding of animal welfare principles among all staff.

Finally, by reviewing amendment requests for ethical projects to ensure that changes do not negatively impact animal welfare, efficient AWB facilitates a more responsive process and eases the burden on ethics committees.

It is therefore evident that the empowerment of AWB is of paramount importance for the fostering of a robust culture of care and the safeguarding of animal welfare in research. It encompasses the dissemination of best practices and knowledge, for the embedding of a philosophy of care throughout the institution, thereby enhancing research integrity and ethical standards.

PD008

Being the Local Ethics Committee of a CRO: How to Overcome the Challenges

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Abstract
Ethics committees (ECs) play a crucial role in overseeing the ethical use of animals in research, focusing on reducing harm, applying the three Rs and balancing potential benefits against animal suffering. The main mission of a local EC in its specific institute is to issue a reasoned ethical opinion on the scientific projects proposed to them by estimating the expected benefits of the research while ensuring that the animals are protected. It involves fundamental principles such as independence, confidentiality and allowed time to overview the potential impacts of the project for the animal. Being the local EC of Contract research organizations (CROs) can strongly complicate application of some of these principles. In this presentation, we will review the challenges we are facing as the local EC of a CRO such as the respect of confidentiality for our external members, the management of the possible conflict of interest for our internal members, the communication with the scientists to have the holistic view of their project before it is implemented or the gap between the time needed for the ethics committee to accept a project and the desire of the CRO to meet the scientist's needs as quickly as possible. We will also give some concrete examples we implemented internally to overcome these challenges. Understanding the potential problems an EC might

face and possible solutions provide the tools for assessing and improving performance of committees and, at the end, promoting animal welfare and ethical standards while doing better science.

PD009

Exploring Workplace Well-being and the Empowering Potential of Creative Writing in Hellenic Vivariums' Personnel

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Abstract
Job satisfaction has been studied amongst laboratory animals professionals in research leading countries. Emotional challenges like compassion fatigue and burn-out have been reported and several strategies to face them are suggested. The 'Care-full Stories'1 where participants share their own narratives from working in animal research, inspired us. Therefore, we aimed to explore for the first time the factors impacting workplace well-being of hellenic vivariums' personnel and its receptivity to creative writing as an empowering technique towards emotional resilience.

An anonymous online questionnaire was addressed to 50% of country's vivariums managers and the vivarium's staff participation was requested. 37 answers were collected predominantly from researchers, managers and technicians.

Our descriptive survey showed that 80% of the participants experienced work stress. Demographic factors like age and occupational experience didn't affect symptoms variety. Anxiety and impaired concentration were notably reported by women. Sense of duty, relief and sadness were mainly reported when applying euthanasia. Interpersonal miscommunication, exhaustion due to solitary work, meet deadlines, perform euthanasia and inefficient infrastructure were the major work stressors. Mental counseling services were suggested as important provision to employees in modern times.

The most frequently applied positive stress-management techniques were engagement in hobbies and conversations with colleagues. Surprisingly 95% of the participants expressed willingness to use creative writing as a self-awareness tool and 45% were interested in using it for communicating animal research mainly in a comic book format or tailored educational programs for school students.

PD010

Quality of Life in Laboratory Animal Professionals: Fatigue and Satisfaction in a Brazilian Institution

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Abstract
Recognition of the occupational risks to the mental and psychological health of laboratory animal professionals (LAP) are not already established in Brazilian research institutions. An important consequence of these risks is compassion fatigue, a profound physical and emotional exhaustion resulting from working in an environment of intensive caregiving (La Follete et al., 2020). This study aimed to analyse the quality of life of LAP working in a public health institution in Brazil and how it relates to the working context. An online questionnaire was addressed to 165 LAP to measure Professional Quality of Life (ProQOL) and several sociodemographic and work-related variables. To this end, a descriptive analysis and multiple exploratory analyses were conducted. The scores of Burnout ($M = 22.6$; $SD = 5.6$) and secondary traumatic stress ($M = 21.1$; $SD = 6.2$) were below the estimate for compassion fatigue. The level of compassion satisfaction was elevated ($M = 39.3$; $SD = 6.1$) suggesting that they felt engaged and fulfilled with their work. We also identify potential protective factors such as environmental enrichment practices (95.8%), humane animal handling (64.2%), human-animal interaction (pet $= 53.3\%$; talk to $= 53.3\%$; observe $= 63.7\%$) and social support (68%). General linear model analysis suggested that euthanasia with inhalant anesthetics ($p = 0.021$) was associated with lower levels of compassion fatigue and maybe a predictive factor. The results will support the development of an institutional mental health care program aimed at preventing compassion fatigue through specific coping strategies for these professionals.

PD011

Advancing Animal Welfare and Ethical Research: 3R and Culture of Care Policy at UNIGE

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Abstract
For several years, the rectorate of the University of Geneva in Switzerland (UNIGE) has been committed to implementing a policy based on the 3Rs (Replacement, Reduction, Refinement) and the "Culture of Care" principles. However, as often in academic institutions, the implementation of animal research policies lies within faculties and departments. Then, our challenge has been to connect policy makers and implementers, namely the rectorate and the community of researchers, in order to improve the welfare of animals used in research and breeding, as well as the working conditions and well-being of animal facility staff. Here we present

the steps that we have undertaken towards this aim. (1) Policy on transparency: by joining the Swiss Transparency Agreement on Animal Research, the rectorate engaged in supporting researchers with their communication activities; (2) Focus on personnel wellbeing: a dedicated taskforce was created to convene animal facility, human resources, and health safety managements to tackle together working conditions and well-being of animal care staff; (3) Promoting education: providing researchers with targeted training on the 3Rs, advanced animal monitoring methods, and the preparation of animal experimentation applications; and (4) Exceeding legal animal welfare standards: implementation of a rehoming program for research and breeding animals as well as initiatives to reduce breeding surplus. Through these measures, UNIGE continue to seek to promote ethical research practices, enhance collaboration, and set an example for sustainable and responsible management of animal research in academic institutions.

PD012

Learning from Failure: Fostering a Culture of Care

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

In this talk I will present the journey of setting up a "No-Blame, Learning from Failures" system in AstraZeneca. To do this, we adopted the "Human and Organizational Performance" (HOP) mindset from workplace safety and used it in the context of culture of care. We developed a "learning-log" and a method to address and take learnings from errors effectively. The log is an automated process that includes an easily accessible event reporting tool called "Learning Log" and a Root Cause Analysis practice. I will discuss our learnings (pros, cons, requirements) and provide advice on how to address this new way of working in trustful collaboration with staff.

PD013

Integrating Animal Care Staff and Laboratory Technicians in Research: Researchers' Perspectives

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Abstract
Animal research has significantly advanced human health, contributing to scientific breakthroughs. However, concerns over reproducibility persist, with low reproducibility rates affecting experimental reliability (Baker, 2016). Addressing these challenges may require greater involvement of animal care staff and laboratory technicians in experimental planning and implementation. Their expertise in animal welfare, behaviour, and housing conditions is crucial for improving research quality, minimizing variability, and assuring accurate results.

This study analyzed open-ended survey responses from researchers about the roles of animal care staff and laboratory technicians in experimental planning and implementation, highlighting professional interactions, challenges, and suggested improvements. Data were analyzed using word frequency analysis with R software and inductive thematic analysis supported by computer-assisted data analysis tools.

Responses highlighted "animal," "staff," and "care" as dominant words, reflecting the central role of animal welfare and staff. Other key words included "time," "technician," "experiment," "train," "facility," and "interaction". Thematic analysis revealed that professional interactions are often person dependent, with rigid professional hierarchy, turning collaboration and positive experiences challenging. While some researchers advocated for including animal care staff in experimental discussions, others resisted this approach.

The findings point to key challenges and areas for improvement, with researchers claiming better infrastructures and training improvement for caretakers. Effective communication was highlighted as the central pillar for fostering a positive work environment, and for improving research outcomes.

Acknowledgments: This work was conducted within the COST Action IMPROVE ("3Rs concepts to improve the quality of biomedical science"), CA21139, supported by COST (European Cooperation in Science and Technology).

PD014

Do We Really Communicate? Improving Communication among Stakeholders in Animal Research

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Abstract
Background: Effective communication among stakeholders is essential in animal research to warrant ethical compliance, animal welfare, workers satisfaction, and high-quality experimental outcomes. Stakeholders, including animal care staff, laboratory technicians, facility managers, veterinarian, and authorities or ethics committees, play important roles in planning and conducting animal experiments. However, communication gaps among these groups can lead to inefficiencies, reduced collaboration, and ethical concerns.
Material and Method: Survey responses from stakeholders involved in animal research were analysed to assess communication levels and their involvement in planning and conducting experiments. Communication and feedback frequency were analyzed using a five-level Likert scale whereas recognition was evaluated using a four-level scale.
Results: Communication of researcher was ranked highest with laboratory technicians and animal care staff, with "good" and "outstanding" levels dominating. In contrast, communication with veterinarian and authorities showed higher proportions of "no" and "little" communication. Animal care staff and laboratory technicians were more involved during the conducting than during the planning phase of animal experiments. Feedback meetings occurred inconsistently. Feedback with lab technicians occurs more frequently than with animal care staff. Recognition practices in acknowledgments is different for lab technicians and animal care staff.
Conclusion: This study indicates gaps between researchers and veterinarian. Hence, it underlines the need for targeted efforts to improve communication with veterinarians and authorities about animal experiments. Moreover animal care staff and laboratory technician's involvement in planning animal experiments should be encouraged,
Acknowlegments: This work is done within the IMPROVE (CA21139), supported by COST (European Cooperation in Science and Technology).

PD015

„ZPF-Voices": Let ALL People Speak!

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Abstract
„Culture of Care "is of high interest in laboratory animal science for all professions. Especially animal caretakers are often exposed to high levels of emotional stress. This can lead to dissatisfaction with the job and also compassion fatigue, which then may negatively impact animal welfare.

Animal caretakers are often forgotten when it comes to their involvement and have "no voice" to shape internal processes and improve their everyday work, although they are an indispensable part of every laboratory animal facility enabling high-quality research.

To give the animal caretakers a voice, we at the Center of Preclinical Research of the German Cancer Research Center in Heidelberg initiated the new committee "ZPF-Voices" at the end of 2022.

"ZPF-Voices" is composed of 9 animal caretakers, who represent all animal caretakers, and two veterinarians. All animal caretakers have the possibility to submit topics to the meetings that need to be addressed to improve workflows and the overall working atmosphere. Additionally we implemented an external coaching of all animal caretakers, where they learned how to resolve conflicts in their teams and establish a good team atmosphere.

Approximately two years after the start of "ZPF-Voices" we can draw a positive balance. Together we were able to make some positive changes and improve the working atmosphere, which was, among other things, reflected in a lower number of job terminations among the animal caretakers.

These positive changes not only affect the well-being of the employees, but also animal welfare, which is indispensable to allow responsible research.

PD016

In Vivo Openness for Everyone, by Everyone

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Abstract
Traditionally, guided tours of our In Vivo facilities were conducted by a limited number of individuals, restricting the scope and adaptability of the experiences offered. Our new approach democratizes this process by empowering anyone involved in In Vivo—from Scientists to Animal Technicians and Operations staff—to host visitors. This shift allows for personalized tours tailored to the interests and needs of diverse audiences, ranging from educational groups to technical professionals.

The visitor corridor is transformed to support a variety of guiding activities to accommodate tours for youth education about In Vivo science, detailed explanations of technical operations, and live demonstrations of specific models and procedures. Key upgrades include interactive touchscreens, conference screens, and engaging printed graphics, such as cut-out life-sized pigs and informative signage. Additionally, a wealth of multimedia resources, including videos, presentations, and posters, have been developed or is the making to support these activities. A standardized script for hosting tours is also created to support a wider range of guides.

Our visitor's corridor feature clearly visible areas such as surgical suites for both rodents and pigs, an inhalation lab, transgenic mouse model creation labs, animal holding rooms, and the cage wash area. The visitor experience is enhanced by two operational states: a guided state, where tour guides lead the content, and a steady state, where visitors can independently explore the corridor, interacting with smart signs and observing live activities.

This initiative fosters an environment of transparency and engagement, making In Vivo science more accessible and understandable.

PD017

Establishment of a National Training Program for Trainees and Technicians

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Abstract
Introduction: The continuous training of trainees and technicians in the field of laboratory animal care is essential to ensure quality and ethical standards in animal-assisted research. To meet this need, a Germany-wide, online-based training course has been initiated, which takes place every two weeks. This initiative aims to close the gaps in regional training and create a uniform knowledge base.
Implementation: The training are organized via the Zoom platform and are aimed at trainees and technicians from various companies and institutions throughout Germany, Austria and Switzerland. The courses include topic-specific lectures, interactive discussions and practical instructions delivered by experienced animal technicians, animal house managers, veterinarians and other professionals. Participants are also encouraged to share their own experiences and ask questions about current challenges.
Conclusion: The Germany-wide training have established themselves as a valuable resource for the training and further education of apprentices and technicians. The online-based structure enables flexible participation and promotes exchange between the various institutions. Initial evaluations show a high demand for this additional knowledge transfer and promotes a significant improvement in participants' technical knowledge and confidence in their professional practice. This initiative makes a significant contribution to harmonizing standards in laboratory animal care and expanding the professional network of participants.

PD018

One Welfare Committee, Integrating the Welfare of Animals, People and the Environment

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Abstract
Breeders have no regulatory obligation to set up an ethics committee, but some have embraced the practice to uphold high animal welfare standards and obtain accreditation. Bioculture is a company dedicated to the ethical breeding and export of Mauritian cynomolgus monkeys for biomedical research. The company was founded in 1984 and has been AAALAC accredited since 1999. An ethics committee, in line with an IACUC, has existed since then but was revised in 2023 to advance an integrated approach to welfare.

The One Welfare Committee (OWC) is a decision-making body whose role consists of setting, implementing and monitoring standards of animal welfare, human welfare and environmental sustainability across the company. 'One Welfare' is an extension of the One Health concept which posits that animal welfare depends on and influences human welfare and environmental sustainability.

Led by a multidisciplinary team, the OWC actively promotes better consideration for, and continuous improvement of, animal welfare, human welfare and environment/planet welfare among employees. Corporate Social Responsibility initiatives such as community support programmes and investments in environmental protection (beyond site environmental management), whilst not directly linked to the works in animal facilities, add an extra dimension to 'One Welfare' and propose an innovative approach to societal commitments.

This presentation will discuss the merits and challenges of adopting an integrated welfare model within research and breeding operations. By sharing the OWC's early outcomes—such as policy advancements and best practices—this talk aims to shed light on the operationalization of the One Welfare concept.

PD019

Fostering a Culture of Care: Integrating Compassion and Collaboration within a Positive Research Culture

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Abstract
Fostering a Culture of Care: Integrating Compassion and Collaboration within a Positive Research Culture

Caring for animals used in research is not only the right thing to do but also helps produce better scientific results. This poster explores how fostering a Culture of Care within a positive

Research Culture can improve animal welfare, strengthen ethical standards, and enhance collaboration in research institutions.

A Culture of Care refers to an institutional commitment to high animal welfare standards, ethical research practices, and a supportive environment for staff involved in animal research (Howard et al., 2016; Franco & Olsson, 2014). Assessing a Culture of Care can be achieved through surveys, welfare audits, and staff engagement initiatives (Hobson-West & Davies, 2018).

We share practical ways to build empathy and responsibility, such as training programmes to improve animal care skills, recognising staff who show compassion, and encouraging teamwork between researchers, veterinarians, and animal technicians.

The poster also highlights the importance of creating a workplace where staff feel safe to speak up about welfare concerns and suggest improvements. We'll share real-life examples from successful programmes that have improved animal care and strengthened staff morale.

Following recent changes in senior management at our establishment, our goal is to inspire FELASA attendees to advocate for care and compassion, ensuring that laboratory animal research upholds the highest ethical standards and scientific quality.

PD020

A Global CRO Strategy to Foster a Strong Culture of Care

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Abstract
Simply stated, a Culture of Care encompasses research animal welfare, employee welfare, scientific quality and integrity, and openness and transparency. Each aspect of a Culture of Care reinforces the other and represents an institutional commitment by each facility, team, and individual. Charles River Laboratories has cultivated a strong Culture of Care across a global organization through multiple strategic initiatives including enhanced communication, training, acknowledgment of individual contributions to science through implementation of the 3Rs, mechanisms for addressing concerns, and fostering openness and transparency through internal and external outreach. Attendees will learn how these programs have had a demonstrably positive impact on the Culture of Care at >70 sites, across multiple regions and cultures. Participants will gain insights into the challenges and successes of supporting a Culture of Care across culturally diverse facilities and teams. This will include global training strategies, targeted 3Rs initiatives, recognition programs, employee engagement, and involvement in openness and transparency initiatives. Attendees will discover resources for supporting employee development, implementing 3Rs initiatives, fostering transparency and openness, contributing to enhanced employee satisfaction and animal welfare. All underscoring the vital role of a strong Culture of Care in driving progressive change within the industry.

PD021

Phytoestrogens in Tahini and Sesame Perisperm: Dual Benefits for Human and Animal Skeletal Health?

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Abstract
Phytoestrogens are increasingly favored by the public due to their reduced adverse effects compared to pharmaceutical treatments of osteoporosis. Taking into consideration the multi-benefits of phytoestrogens and their low adverse effects, the impact of sesame [tahini (T) and sesame perisperm (SPE)] consumption was examined on bone mineral density in the ovariectomized (Ovx) rat osteoporosis model.

Four groups of ten-month-old female Wistar rats (control, Ovx, Ovx+T, Ovx+SPE) were studied for 6 months. Tibial bone density values of Ovx+T and Ovx+SPE, measured by Dual-Energy X-ray Absorptiometry, were significantly higher than the Ovx group at 3 and 6 months. The femoral *ex vivo* biomechanical strength test also resulted in statistically significant improved values of maximum bending stress, shear stress, and the equivalent von Mises stress. It was concluded that both tahini and sesame perisperm seem beneficial for the femur bone with sesame perisperm being the most beneficial. These findings suggest that sesame tahini and perisperm can improve tibial bone density and biomechanical strength following estrogen depletion, highlighting the potential of phytoestrogens in managing postmenopausal osteoporosis.

It should be pointed out that results such as these, are beneficial not only for humans but for animals *per se*. While animals do not suffer from postmenopausal osteoporosis, these favourable findings on bone mineral density and strength open the way to explore the potential beneficial effects of sesame consumption on skeletal health. By focusing on the "One Health" approach, the importance of integrated health strategies that benefit both humans and animals should be highlighted and communicated.

PD022

Change Management Improving the Merck 4Rs (REPLACE, REDUCE, REFINE, RESPONSIBILITY) in Contract Research

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Abstract
This talk will share insights and experiences making significant improvements in animal welfare metrics while fostering a culture of care within an established GXP compliant Contract Research Organization using effective change management.

In our initiative, we recognized the need for change driven by evolving market demands and the necessity to enhance our operational metrics related to animal welfare. We began by conducting a thorough assessment of our current state, identifying key stakeholders, and understanding the impact of change on various teams involved in animal care. This groundwork allowed us to develop a comprehensive strategy focused on communication, training, and support.

Central to our approach was engaging stakeholders at every level. We implemented clear communication plans to ensure transparency and foster trust, while tailored training programs equipped our employees with the necessary skills to adapt to new welfare standards and practices. By emphasizing the importance of a supportive environment, we encouraged a culture of care where both employees and supporting stakeholders felt valued and empowered.

Throughout this journey, we measured the effectiveness of our initiatives through key animal welfare metrics, maintenance of business KPI targets and tracking employee engagement surveys, allowing us to make data-driven adjustments. Ultimately, our focus on change management not only improved our operational metrics but also cultivated a resilient culture of care that prioritizes animal welfare and employee well-being. This holistic approach has positioned us to thrive in an ever-evolving business landscape, and I look forward to sharing more about our journey and its outcomes.

PD023

Animal Welfare Boards (OPBA) in Practice: Comparing European and Non-European Approaches

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Abstract
The Animal Welfare Bodies (AWBs) are a significant scientific advance in animal research, introduced by Directive 2010/63/EU. These bodies have an advisory role on various animal welfare issues within research institutes.

AWB tasks include the application of the 3Rs principles (replacement, reduction, and refinement), training of animal facility personnel, animal management at all phases of experimental research, as well as the introduction and review of monitoring and reporting processes in these areas, including animal rehoming programmes.

The Directive makes the evaluation of research projects involving animals mandatory, but does not explicitly entrust this evaluation to the AWBs. Nevertheless, in many Member States, AWBs of user establishments are responsible for an initial assessment, which requires involving external actors to ensure adequate expertise and impartiality.

This study compared AWBs from various Member States (France, Germany, Spain, the Netherlands and Italy) and non-European countries, such as the United Kingdom and the United States. The latter relies on the Institutional Animal Care and Use Committee(IACUC) for the care and use of laboratory animals.

Significant differences exist between European AWBs and US IACUCs in structure, role, and approach, influenced by local culture. Both aim to use animals only when necessary, minimizing their numbers and suffering. European AWBs have a more bureaucratic, transparent approach, promoting external consultations to meet public expectations. In contrast, the US system relies on self-regulation and trust in researchers, with less focus on bureaucracy or punitive measures for non-compliance.

PD024

The NHP-specific FELASA Course: Practical Training in a Skills Lab

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Abstract
The work with non-human primates (NHP) in research is challenging and personnel need a highly specialised and thorough education. To meet this challenge, the network of European primate centres (EUPRIM-Net) developed an NHP-specific Laboratory Animal Science course. Since 2017 the NHP-LAS course is accredited by the FELASA for Directive Functions A & B and offered twice a year by the German Primate Center (DPZ). To our knowledge this is to date the only FELASA accredited NHP-specific LAS course in Europe.

The course is comprised of an e-learning part and an on-site part taking place on-site at the DPZ in Göttingen, Germany.

Since it is not permitted to use NHP for education purposes, participants learn amongst other practical contents: behaviour monitoring, sterile dressing, and the principles of clicker training. Since 2024 we have integrated a Skills Lab into the course where participants can try out several procedures (such as blood draw, suturing, transportation of marmosets, squeezer cage, implant care, etc…) on appropriate models. Within the frame of the course, training videos are offered for handwashing and suturing. For further practical skills, these are obtained after the course in the individual laboratories when handling NHP under supervision.

PD025

Inform – Connect – Innovate The Way of the Austrian 3R Center

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Abstract
The Austrian 3R Center (A3RC), established in 2020 by the RepRefRed Society and funded by the Federal Ministry of Education, Science and Research, serves as Austria's national knowledge platform for the 3Rs and related topics. Aiming to set up a strong national and international 3R community, A3RC organizes initiatives such as the Austrian 3R Days, online seminar series, and active engagement through social media. Beyond uniting the 3R community, the A3RC emphasizes the importance of connecting smaller national stakeholder groups, including animal welfare bodies in academia and industry, as well as laboratory veterinarians in Austria. The A3RC regularly organizes meetings for the animal welfare bodies and the laboratory veterinarians to facilitate knowledge exchange among these groups. Initially, the A3RC knowledge transfer mainly focused on scientific staff as its primary audience. However, recognizing the importance of inclusivity, the center is now expanding its efforts to engage a very important group of people, the animal caretakers across Austria, by setting up an exclusive online seminar series for animal caretakers. By establishing these networks, the A3RC is able to completely connect all the national game players in animal research with each other. This offers the opportunity to have a network all over the animal research landscape in Austria to distribute information in a more targeted way, which will drive forward innovation in the field of the 3Rs.

PD026

Designing Mental Health Program for Animal Researchers

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Abstract
The number of laboratory mice and rats used in Korea is about 3 million per year [1], and the psychological trauma of researchers conducting animal experiments is a concern in this country. Accordingly, it is necessary to prepare a mental health program to prevent post-traumatic stress disorder in animal researchers [2]. In this study, the stress of animal researchers was analyzed through an online survey and an in-depth interview of purposeful samples, and a mental health program model for animal researchers was developed through a comparative study with similar occupational groups. About 40% of the respondents felt nervous and overwhelmed by their surrounding environment due to their job, suggesting a risk of emotional exhaustion and that continuous support was needed. Based on our investigation, we recommend a mental health program as follows: 1) Pre-training step: It is a stage in which workers increase their practical understanding and promote vocational awareness through pre-training before they are put into the job 2) Prevent step: Promote the well-being of researchers as a precautionary measure, and support emotional exchange through interaction with animals 3) Detection step: When a researcher complaining of stress is detected, policies such as flexible working hours operation are used to support workers to take appropriate breaks and provide training based on the 3R program 4) Care step: Provide free conversation groups for psychological counseling and emotional support between colleagues. It prevents employees from being emotionally exhausted, increases job pride, and supports them to form a positive job attitude.

PD027

Rodent Euthanasia: The Role of Animal Welfare Expert in Soothing This Emotional Burden

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Abstract
Compassion fatigue is a deep feeling of physical and emotionnal exhaustion that can appear while working with laboratory animals and witnessing animal suffering. There is an increase in awareness about this issue and how wildspread and frequent it is in our work. Yet, it remain one of the main causes of high turn over in animal experimentation, and solutions can be hard to identify and implement.

We struggle to find the right balance between rationality and emotions, as being "too emotional" is often seen as a risk of bias in the scientific environment. We are judged by ourselves, our colleagues and our peers. Veterinarians and animal welfare experts have an important role to play here. By bringing their scientific, behavioral and welfare expertise, paired with their animal suffering empathy, they can become a bridge between rational and emotional people. They can help bring understanding of one another and appeased decision making on both sides, decreasing the burden of ethical dilemmas by sharing it.

Euthanasia is a perfect illustration of this challenge, systematically appearing as a major cause of compassion fatigue. Making hard decisions, especially in a context of urgency, can lead to emotional strains and doubts about the rightness of our choices, our actions. This presentation will follow three cases study (planned euthanasia, end point reached, depopulation) and tackle the ethical dilemma they generate, their consequences on everyone involved and will suggest practical solutions to reconcile the brain and the heart, to improve our methods and communication on this subject.

PD028

Symphony of Compassion: Ethical Treatment of Animals in Scientific Inquiry

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Abstract
"Symphony of Compassion" represents an interdisciplinary approach to fostering care, openness, and ethical conduct in animal research. Just as a symphony harmonizes diverse instruments into a cohesive work, we must align our efforts to advance scientific knowledge while ensuring the welfare of sentient beings. Our presentation follows the structure of a symphony, with five key "movements" that build toward a shared vision of progress.

The first movement, *Ethical Framework*, establishes the foundation with principles such as prioritizing welfare, avoiding harm, and adhering to ethical guidelines like the 3Rs (Replacement, Reduction, Refinement) and IACUC oversight. The second movement, *Scientific Rigor and Openness*, emphasizes reliability and transparency through rigorous methodologies, pre-registration of studies, open-access publishing, and data-sharing platforms. The third movement, *Cultural Shift*, focuses on transforming attitudes and practices through education, outreach, and empathy-driven collaboration, with initiatives such as ethical treatment training and internal awareness campaigns. The fourth movement, *Public Engagement and Dialogue*, highlights the importance of constructive conversations with the public, expanding transparency beyond the lab, fostering societal participation, and engaging in partnerships with community organizations. The final movement, *Innovation and Alternatives*, explores forward-looking approaches to minimize or replace animal use while advancing scientific discovery. By emphasizing innovative methodologies and alternative models, we can align progress with ethical commitments.

Together, these movements form a "Symphony of Compassion"—a harmonious, ethical approach to animal research that balances scientific advancement with care and responsibility for all living beings. Through collaboration and dedication, we can create a future rooted in compassion and discovery.

PD029

Enhancing a Culture of Care Through Animal Welfare Teams, Event Recognition, Reporting, and Analysis

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Abstract
As institutional culture of care has matured, there is growing awareness of the need for self-recognition of animal welfare events to optimize animal well-being and to ensure transparency of processes for personnel. Defining events to be reported, training personnel, and developing an outcomes-based process for robust root cause analysis and development of corrective and preventative actions are all necessary steps in the process for managing and preventing future events. Creating an animal welfare team, with personnel identified as animal welfare specialists, facilitates event reporting, analysis, and follow up. Through the team approach, transparency and openness around animal welfare events further enhances the culture of care at an institution. This session will provide examples of (1) how institutions can develop internal animal welfare reporting programs, and (2) how using animal welfare teams and a process-focused approach with animal welfare issues and near misses can help to drive improvements in animal welfare and overall institutional culture of care. The target audience includes technicians, facility managers, compliance specialists, and veterinarians.

PD030

Promoting Humane Research Practices: Transitioning to Non-Aversive Handling Methods for Improved Animal Welfare

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Abstract
The term "Culture of Care" refers to an organizational commitment to improving animal welfare, scientific quality, and the well-being of staff involved in biomedical research projects. Numerous studies demonstrate the link between the care provided to animals and the quality of life of laboratory personnel, often resulting in better research outcomes. Additionally, mice handled using non-aversive methods, such as tunnel handling instead of tail handling, exhibit less anxiety and aversion during human interaction or when exposed to new environments.

The CMCiB implemented the Culture of Care by replacing tail handling with tunnel handling in its rodent animal facility. A comprehensive plan was put into action, which included theoretical training sessions for animal technicians and researchers, emphasizing the benefits of improved handling techniques for mouse welfare. The plan also involved revising the cage-changing Standard Operating Procedures (SOPs), replacing tail handling with the use of cage enrichment (such as tunnels and nest material) during animal transfer between cages, and incorporating practical training on animal handling for researchers.

Feedback was collected through surveys from all involved personnel. Results showed a general consensus that the animals were calmer and more accepting of human interaction. Participants reported greater ease in performing experimental procedures such as handling, weighing, and administering subcutaneous injections, with all staff members acknowledging a positive perception of animal welfare.

These findings demonstrate that by introducing non-aversive methods during cage changing, an organization can effectively implement a culture of care that promotes a more humane and responsible research environment.

PD031

Openness - Facts and Plans to Inform the Lay Public

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Abstract
Animal testing is a kind of negative mysticism in the minds of many people. Lack of information and knowledge lead to misconceptions and misinformation that negatively affect the perception of the research work.

By completing questionnaires anonymously, we explored people's perceptions of animal experimentation and found that in most cases extreme negative associations are attached to it, e.g. murder, suffering, pain. Therefore, our objective is to produce information material, first in the mother language and then translated into English, that factually and accurately explains the importance of animal testing. As our target audience is laymen, the language of our talks and information materials is also non-technical.

As our target audience is the lay public, the language used in our discussions and information material is also non-technical. First we visited a secondary school and talked to young people aged 15–16. We took part in the Researchers' Night, where we organised a programme for teenagers to express their opinions. During the conversations, we were able to find out what they know about animal testing and inform them about the real facts. Our work is also supported by posters on different topics eg. the history of animal testing, the 3Rs, misconceptions, the legal background, and by virtual visiting og some animal facility. A short video entitled 'False Arguments - Truths' has also been produced that received professional appreciation

With an emerging working group, we plan to compile a complex information material that could be a 'gap filling' product in our country.

PD032

Advantages of an All-husbandry-embracing Intramural Animal Welfare Body

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Abstract
The animal welfare body (AWB) is required by the directive 2010/63 EU for breeders, suppliers and users of laboratory animals. However, the implementation and execution can vary greatly between institutions, leading to different impacts on the culture of care. At the Medical University of Vienna, a satellite husbandry based, mosaic like structure of the AWB has recently been supplemented by a comprehensive approach, combining all husbandry facilities in one committee. It has been shown that this has many advantages that will be reviewed here.

- A **large number of participants** from different husbandry units allow **constructive outside reflection**.
- Any **incidents on animal welfare** issues are received by an **impartial party**. The reports are forwarded to the project leader and head of husbandry. All documented cases are included in the final report each year that is sent to the rectorate and can be checked by the authorities.
- This **transparent procedure** leads to a **high commitment** among the people involved.
- In **disputed situations** the AWB forms **working groups, scientifically evaluate** the situation and works out **recommendations**. For example, recommendations for environmental enrichment or medical guidelines to ensure high-level animal husbandry standards.
- **Educational, group building and public relation actions** can be coordinated centrally by the AWB. For example: training for animal care staff, which, next to education, also provides a save environment to connect with and learn from each other; supervision groups for animal keepers and veterinarians; guided tours through husbandries for medical university staff.

PD033

Guided Tours in a Laboratory Animal Husbandry Positively Change the Attitude Towards Animal Testing

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Abstract
On 24.4.2024, World Day for Laboratory Animals, the Center for Biomedical Research opened its doors to give MedUni Vienna employees an insight into a laboratory animal husbandry. The tour aimed to offer transparent information on animal testing, especially focusing on husbandry conditions. Accompanied by the tour, a survey was conducted, asking the participants about their general opinion on animal testing, their expectations of the husbandry conditions as well as their opinion of the personnel working with laboratory animals before as well as after the tour. In general, it was asked about their opinion on transparent information about animal testing.

3 tours of 15 people each were conducted and 30 people completed the survey. A tour duration of one hour per tour was planned, but this was significantly exceeded due to the lively interest in each individual tour. The tours were led by the veterinarians responsible and consisted of a conventional mouse room, the rat husbandry, finishing at the pigs unit. Highlights were the alternative rat housing system of the Medical University of Vienna as well as the pigs that showed their curious, open and playful character, actively approaching the visitors.

The results of the survey were very positive, all parameters were assessed significantly more positive after the tour than before. Especially the opinion on the personnel working with laboratory animals changed drastically after the tour. This clearly shows that lay people are not only very interested but also open about animal testing if approached in an open and transparent fashion.

PD034

From Adversaries to Allies: Building Stronger Bridges Between Researchers and Veterinarians in Animal Research

N.E. Trimmel[1]
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Abstract
In many animal research facilities, tension often exists between researchers and on-site veterinarians, with veterinarians sometimes perceived as burdensome regulators rather than collaborative partners. This perception can lead to underreporting of animal health issues, missed opportunities for early interventions, and compromised study outcomes. This adversarial dynamic undermines the core principles of a Culture of Care, where staff well-being is prioritized to create an environment in which animal welfare and scientific quality can also thrive.

This presentation will focus on transforming these adversarial dynamics into productive, supportive partnerships that prioritize both animal welfare and scientific integrity. By sharing practical examples and illustrative anecdotes, I will explore strategies to encourage open communication and foster a culture of shared responsibility. These strategies include but are not limited to joint meetings, feedback mechanisms, peer influence, leadership engagement, and in-house training aimed at bridging knowledge gaps and enhancing mutual respect. Furthermore, I will discuss the implementation of collaborative reporting systems for adverse events, designed to empower researchers to report issues without fear and ways to celebrate successful collaboration.

Attendees will leave with actionable insights into how to overcome communication barriers and reframe veterinarians' roles as invaluable allies in achieving research excellence. This shift not only enhances compliance but also reduces stress among staff and ensures animals receive the highest standard of care.

Ultimately, by strengthening the researcher-veterinarian partnership within a framework of culture of care, facilities can cultivate an environment where collaboration drives better outcomes for animals, staff, and the institution as a whole.

PD035

"It's an Ant's Work": A View from the South on Culture of Care

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Abstract
Animal use in research was regulated in Brazil in 2008, and as a result, veterinarians were assigned shared responsibility for laboratory animals. This study explores the views of Brazilian veterinarians working in animal research facilities, examining the challenges they face in seeking to foster Cultures of Care. In 2023, in-depth interviews were conducted with 24 veterinarians covering the five regions of the country. The interview transcripts were inductively coded and four main themes were identified. Participants expressed concerns about (i) gaps in training, (ii) infrastructure and maintenance challenges, (iii) resistance to new care approaches, and (iv) barriers in communication. Additionally, it was evident that social dynamics within research facilities, which are characterized by institutional hierarchies and power asymmetries between professorship positions (sometimes occupied by senior researchers) and technical roles (many of which are held by women and mixed-race individuals) create obstacles to the development of Cultures of Care. More positively, participants shared their experiences in improving animal welfare, showing that despite the challenges, they found scope to gradually improve care practices. This movement is constructed through the metaphor "*trabalho de formiguinha*" (It's an ant's work), meaning collective and individual efforts combined for long-term change. These findings suggest, firstly, that animal research in Brazil has been going through a reconfiguration as a result of the recent regulatory act; and that secondly, greater efforts are necessary to foster Cultures of Care through the development of institutional governance policies that address the need for investment in infrastructure and counter-balance power inequalities.

PD036

Podcast Coadjuvantes da Ciência: Exploring Plurality in Animal Research Communication

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Abstract
The use of animals in research has been regulated in Brazil since 2008, marking a shift toward improved animal care and the development of professional standards in the field. Additionally, the Culture of Care has gained prominence in South America, with effective communication among stakeholders being essential to fostering caring environments. In this abstract, we introduce the podcast *Coadjuvantes da Ciência* (Supporting Actors of Science), a local initiative designed to promote ethical reflection and improve communication about animal research in the Portuguese language. Podcasts have become a valuable tool for scientific dissemination and educational support. In this digital media, informational content is delivered through audio files, emphasizing oral communication and listening. We propose a pluralistic approach, which embraces the coexistence of conflicting values, to bridge the gap between universalist and relativist perspectives, improving social relations through mutual respect and understanding. Thus, attentive listening and respect for different views can enhance communication about care. The first season includes a short introduction and eight interviews covering topics such as (i) Brazil's regulatory framework, (ii) animal welfare science, (iii) laboratory animal science, (iv) the role of animal welfare associations, (v) animal rights, (vi) utilitarianism and humanism, (vii) public health, and (viii) alternatives to animal use. To date, there have been 656 streams recorded on Spotify and 1,094 followers on Instagram. In summary, *Coadjuvantes da Ciência* offers diverse perspectives in an accessible audio format, serving as a tool to encourage active listening and ethical reflection on animal use.

PD037

Uplifting Culture of Care for Animal Welfare

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Abstract
Culture of care refers to going above and beyond the legislative requirements to optimise animal welfare and the wellbeing of the people working with them. Under this guiding principle, AST Gothenburg site aims to further refine rat housing environment and handling methods. The standard housing for rats in the Gothenburg vivarium is a large three-tiered house, originally designed for rabbit housing (Figure 1). These compartments, according to AAALAC specifications, are a high standard of rat housing allowing rats more opportunities for natural behaviours such as climbing, hiding, running, and rearing. It is established that boredom in laboratory animals should be addressed1, and that rats prefer a complex environment2 . To add complexity to their environment, an adjustable lift is used to position an extended play area with free access from their home compartment. The play area consists of varied enrichment designed to encourage natural curiosity and provides a playtime between study procedures. After familiarising rats with the open play area, the handlers do not need to pick the rats from their home environment, which can often mimic chasing them and eventually become synonymous with stressful procedures. The open area also allows better interaction and assessment of the animal wellbeing. Any small changes in their behaviour can be noticed and actions can be taken sooner. Importantly, it has been observed that rats are less stressed by handling and study procedures and choose to stay in the play area, which is a good indication that it has positive associations for them

PD038

Importance of Emerging Agent *Burkholderia* Sp. as Cause of Neurologic Symptoms in Immunocompromised Mice

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Abstract
NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice are intensively used in our animal facility as an *in vivo* model to study cancer and develop innovative anti-cancer therapies, which translate into the clinic. Lately, our animal care staff observed an increased incidence of neurologic signs in our experimental NSG population, such as head tilting. These signs were not related to experimental procedures, administered intervention therapies, or breeding stock population. Histopathological investigation revealed prominent suppurative inflammation centered in the middle ear in many animals, with extension into brain and/or spinal cord cavities in several cases, and often with secondary involvement of atlantoaxial, knee, and/or hip joints with consistent features. Occasionally, other organs such as spleen or kidney were also affected, suggesting systemic spread of an infection.

Culture of ear and oropharyngeal swabs revealed *Burkholderia cepacia* complex by MALDI-TOF mass spectrometry in several animals with a clinical history of neurologic signs, and confirmed *Burkholderia gladioli* by PCR in carrier animals. *Burkholderia* sp. has been reported previously to cause neurologic disease in highly susceptible and immunocompromised strains such as NSG, but not in immunocompetent mice (1). We are currently making inventory of affected animals within the contained area, and identifying the most likely source of infection and spread.

Our case report highlights the importance of institutional culture of care regarding animal health and welfare, and the severe disease symptoms in immunocompromised animals stresses the need for a specific PCR assay for *Burkholderia* sp. in screening programs of immunocompromised mouse strains.

PD039

Introducing Low Stress Handling in an Oncology CRO: Strategy and Impact on Staff

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Abstract
Phasing out aversive handling of laboratory animals is a major topic in the Culture of Care complex. Making the transition to low stress handling (LSH) can be challenging, and staff concerns may range from an increase in time needed for procedures to higher bite risk or even questioning of the benefit to the animals.

At our facility, we implemented LSH of mice over the course of a year through a pioneer team of animal care and procedure technician staff. Staff explored the range of products available to facilitate LSH, such as tunnels in different shapes and materials, as well as various handling techniques (tunnel handling, cupping, scooping) before deciding on a preferred combination. The pioneer team then trained their colleagues, leaving each person to choose the technique and material according to their task or personal preference.

A survey conducted after LSH was fully implemented illustrated the initial doubts but also showcased the shift in mindset of the entire team towards a more positive view of the change. However, the survey also highlighted lingering doubts and challenges that will require further attention, such as modifications for handling untrained or agitated animals or a possible negative effect on resilience because of the more compassionate handling of the mice.

For long term success, it appears crucial to not only have the full support of institutional management but also to involve affected staff in the transition process from the start in order to achieve broad acceptance, including from initially skeptical staff members.

PD040

The Italian Animal Welfare Bodies Network: Enhancing Collaboration for Animal Welfare

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Abstract
Several countries have established national Animal Welfare Bodies (AWBs) network, enabling AWBs to share good practices to define effective processes and fulfill their responsibilities. These networks have significantly enhanced animal welfare, the implementation of the 3Rs, the quality of scientific research, and Culture of care. However, organized AWBs networks are not yet widespread across all EU countries.

Launched by the National Committee for the protection of animals used for scientific purposes, the Italian AWBs network (CIO) started in January 2025 after a one-year work aimed to connect and bring together a wide number of AWBs from academic, private and public research institutes. The network currently comprises 84 AWBs, is supported by a steering committee that meets annually with the National Committee and provides input and co-organize meeting for each of the three geographical groups of AWBs (North-Centre-South Italy).

The CIO collaborates closely with the National Committee, focusing on 1) promotion of the 3Rs; 2) providing topics of discussion and clarification to competent authorities; 3) dissemination of good practices; 4) facilitating information exchange to ensure a harmonized approach at the national level; 5) offering guidance on specific topics related to animal welfare.

Although the AWBs network has only recently been established, its development process and early implementation already demonstrate the perceived need for a coordinated national approach, offering useful insights and practical tools for countries aiming to establish or strengthen their own AWBs network. Future directions include fostering closer collaboration with ENAWB to further advance animal welfare standards across Europe.

PE001

Artificial Intelligence and Comprehensive Management of Animal Facilities: Advantages and Future Outlook

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Abstract
Advances in artificial intelligence are growing in management of animal facilities field by enhancing efficiency, supporting animal welfare, and ensuring ethical compliance. Acting as a complement to human oversight, AI is a valuable tool in biomedical research.
Advantages:

- Monitoring: AI-powered systems track animals' health and behavior, providing early warnings of stress or illness, enabling timely operators' interventions, and improving research quality.
- Operational Support: AI software streamlines improve stocks managements, scheduling, and compliance monitoring, reducing administrative work and freeing personnel for specialized tasks.
- Data Management: AI processes complex datasets, delivering detailed analyses that improve decision-making while relying on researchers to verify findings.
- Ethical Advances: AI facilitates reduced animal usage through advanced modeling and simulations, decreasing reliance on live animals without compromising research validity.

Future outlook:

- Improving Human Roles: AI will support human expertise in animal care and research management, leaving more time for new learnings in the operators and researchers.
- Routine Automation: Robotics and AI will handle works like feeding and cleaning, reducing errors while retaining human oversight.
- Enhanced Ethical Practices: Innovative AI solutions will reduce live-animal studies, leveraging virtual modeling while ensuring ethical and accurate research.

AI's integration into animal facilities offers transformative potential. Getting collaboration between AI and human expertise, the field can achieve ethical, effective, and efficient research while maintaining the essential role of human judgment and care in the animal facilities.

PE002

Estimated PCR Prevalence of Pathogen Contamination in Research Biologics

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Abstract
Cell lines, tumors, and other biologic materials frequently used in biomedical research can be contaminated with infectious agents that could impact research outcomes. As these materials are routinely inoculated or injected into research models, it is vital to demonstrate they are pathogen free to ensure reproducibility of results, avoid health impacts to animals and staff, and prevent the loss of research time and money. Screening for pathogen contamination by PCR prior to introduction into animals is essential to avoid these significant downstream implications. To aide in deciding which pathogens to include in routine biologic screening programs, we mined data gleaned from PCR testing of European and North American samples within Charles River's commercial diagnostic laboratory over the past two decades. These results establish estimated prevalence rates for infectious agents in human- and rodent-derived biologics. *Mycoplasma* were the most common contaminant, with a prevalence of ~5%, regardless of host species. *Corynebacterium bovis* was found in rodent and human biologics, with an estimated prevalence of 1.02% and 1.6%, respectively. Also found in rodent biologics were rodent parvoviruses, LDV, rodent chaphamaparvovirus-1, MNV, Polyomavirus, MAV, Coronavirus, Rotavirus, MCMV, and reovirus, with estimated prevalence values between 0.05% and 0.60%. Human viruses detected in human biologics include EBV, HHV-6, HHV-7, HIV-1, HIV-2, HPV-16, HPV-18, AAV-2, Human parvovirus (B19), HBV, HTLV-1, and HTLV-2 which all ranged between 0.05% and 2.75% prevalence. Notably, LDV, a mouse pathogen, was also detected in human derived materials at a rate of 1.19%, supporting testing of human biologics for rodent pathogens.

PE003

Setting Up an Electronic Animal Management Database (Tips from the Trenches), a Canadian Perspective

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Abstract
Across Canada, many institutions utilizing animals for research, breeding and/or teaching have implemented, or are in the process of implementing electronic animal management databases. Utilization of databases is recommended by the Canadian Council on Animal Care (CCAC) to track animal use, animal health and welfare, CCAC reportable information (Animal use data, RAWIs), 3Rs related processes, and other compliance related issues. In 2017 the UBC Animal Care and Use Program (ACUP) implemented a single database program across multiple sites, health authorities and other locations to ensure consistent and harmonized oversight of animal use across the ACUP. As the second largest ACUP in Canada, extensive consultation was required with all stakeholders to capture the variety of user and facility management needs and UBC Animal Care Committee (ACC)/CCAC oversight perspectives - and many, many tweaks along the way. By sharing the trials, tribulations and successes of our process we hope to aid others with their database implementation, which will allow for better tracking of animal utilization within their programs.

PE004

Barriers and Enablers to Conducting Systematic Reviews at a German University Medical Center

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Abstract
Communities for Open Research Synthesis (COReS) develops a targeted framework to initiate systemic change in how evidence from preclinical studies is translated into improved human health outcomes. Systematic review and meta-analysis are research synthesis tools that advance high-quality research by evaluating how reliable evidence is and clearly identifying knowledge gaps, highlighting where future research is needed.

We employ a three-pillar approach to integrate preclinical systematic reviews into the research pipeline and foster synthesisable primary research practices: i) building capacity with open-access training opportunities and comprehensive online resources for researchers and stakeholders at all experience levels (education); ii) scaling capacity for systematic review conduct by developing tools and software (infrastructure); iii) forging networks through interdisciplinary learning and collaboration opportunities (community).

Here we present our study identifying barriers and enablers to conducting systematic reviews and identify current practices in systematic review conduct at one of the largest European university medical centres (UMCs), Charité – Universitätsmedizin Berlin. We surveyed all UMC staff members about their awareness of and attitudes towards systematic review. We conducted follow-up interviews with interested survey participants examining attitudes and current practices towards systematic reviews.

Our findings highlight specific barriers and enablers identified by multiple stakeholders when conducting systematic reviews at a large European UMC including; lack of awareness of systematic reviews, the large number of resources required hindering systematic review conduct, and facilitatory institutional support. We use these findings within COReS to inform strategies that enact long-lasting change in how preclinical research is designed, conducted, and disseminated considering up-to-date evidence.

PE005

In Vitro and in Vivo Efficacy of New Taxane-based Chemotherapeutics in Pancreatic Cancer

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Abstract
Pancreatic cancer is a severe malignancy with increasing incidence and high mortality due to late diagnosis and low sensitivity to treatments. Search for the most appropriate drugs and therapeutic regimens is the most promising way to improve the treatment outcomes of the patients. This study aimed to compare (1) *in vitro* efficacy and (2) *in vivo* antitumor effects of conventional paclitaxel and the newly synthesized second (SB-T-1216) and third (SB-T-121605 and SB-T-121606) generation of taxane-based chemotherapeutics in *KRAS* wild type BxPC-3 and more aggressive *KRAS* G12V mutated Paca-44 pancreatic cancer cell line models. *In vitro* potency of paclitaxel was 27.6 ± 1.7 nM, while SB-Ts showed 1.7–7.4 times higher potency. Detailed *in vitro* study allowed us to select the most effective taxane-based chemotherapeutics SB-T-121605 and SB-T-121606. Incorporation of SB-T-121605 and SB-T-121606 into the therapeutic regimens of paclitaxel *in vivo* was effective in suppressing tumor growth in Paca-44 tumor-bearing mice at small doses ($\leq$3 mg/kg). The most potent new taxane-based chemotherapeutics *in vitro*, i.e., SB-T-121605 and SB-T-121606, were found to be well tolerated in vivo in combination with paclitaxel, which provides a promising therapeutic regimen for the next phase of preclinical development. This work was supported by the Czech Ministry of Education, Youth and Sports, INTER-ACTION LUAUS23164, the NICR project LX22NPO5102 financed by European Union – Next Generation EU as part of the Czech Recovery Plan, the Grant Agency of Charles University programs Cooperatio no. 207036 and Donatio Facultatis Medicae Tertiae, and the National Institutes of Health (NIH), U.S.A. grant R01 CA103314.

PE006

Utilizing Hematopoietic Stem Cells for Controlled FXN Expression as Gene Therapy for Friedreich's Ataxia

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Abstract
Friedreich's Ataxia or FRDA is a rare neurodegenerative disease, caused by mutations in the FXN gene. As a monogenic disorder, FRDA is a prime candidate for gene therapy.

In our study, we employed autologous, genetically modified-hematopoietic stem cells (HSCs) to achieve a therapeutic outcome. As FXN overexpression has proven to induce cytotoxicity, we first sought to achieve precise regulation of the transgene's expression by using a combination of cis-regulatory elements (CREs).

We developed FXN-encoding lentiviral vectors carrying enhancers with differential potency and cell specificity allowing for expression in the erythroid and myeloid hematopoietic lineage. These vectors were assessed ex vivo in hematopoietic progenitors from mice with an FRDA phenotype (Fxn^{null}::YG8s(GAA)$_{>800}$, Strain#:030395). Specifically, Lineage- bone marrow cells isolated from Fxn^{null}::YG8s(GAA)$_{>800}$ mice, were transduced with FXN-cDNA vectors and were subsequently differentiated towards the myeloid and erythroid lineage. All vectors were able to increase FXN expression in a VCN-dependent manner, without altering the cells' clonogenic capacity or ex vivo multilineage differentiation ability. Of all tested vectors, the combination of an erythroid and a panmyeloid enhancer produced an optimal expression profile with high erythroid and median myeloid expression patterns. Notably, even though FRDA is not known to severely affect the phenotype or function of hematopoietic cells, we did observe a reduction in early apoptotic myeloid cells post transduction. Our results highlight the potential of using genetically modified-HSCs to provide a safe and effective therapeutic strategy for Friedreich's Ataxia, paving the way for future studies on translating this approach into clinical practice.

PE007

Bring the Sociability Test to the Animal, Not the Animal to the Sociability Test

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Abstract
Sex differences in human social behavior stem from biological as well as social factors, with women tending to have closer social networks. These differences vary with life stages and are influenced by sex hormones. In biomedical research, rodent social behavior is often tested briefly outside home cages, potentially causing stress to animals. Continuous home-cage monitoring offers a solution to these limitations. This project evaluated the effects of low estradiol concentrations on the social behavior of female BL6 mice in PhenoTyper cages. Adult females underwent either ovariectomy (OVX) or sham surgery (F). For two weeks, sham-operated female mice were subcutaneously treated daily with either an aromatase inhibitor, letrozole (1 mg/kg, F+LET), or olive oil (1 ml/kg, F), while OVX females received either estradiol (10 μg/kg, OVX+E), or olive oil (1 ml/kg, OVX) followed by open field, indirect, and reciprocal social interaction tests conducted in PhenoTyper cages. OVX females had significantly lower uterus weight than the F females, while OVX+E females showed increased uterus weight compared to OVX females. OVX did not affect locomotor activity or anxiety-like behavior but reduced social disinterest in the reciprocal social interaction test compared to F mice. OVX+E females also showed less social disinterest than F+LET mice. Automated measurements showed no group differences in indirect social behavior, limited by EthoVision XT 10 software. Direct reciprocal social interaction results are preliminary, however, the strengths of this study include using females and combining surgical-pharmacological methods of estrogen deficiency and testing social behavior in a home-cage system. This study was supported by VEGA 1/0341/23.

PE008

The Use of Artificial Intelligence (AI) In Teaching

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Abstract
Artificial Intelligence (AI) has provided us with a range of tools and services to help people with their work tasks. At the UiB we have made a simple start to the use of AI in our laboratory animal work:

Teaching Laboratory Animal Science

Students today have a plethora of AI tools available to help them study. This allows them to adapt and process teaching material in an individual way to suit their own needs and preferences. The challenge for us as educators is to provide quality assured material they can digest using AI. At the UiB we provide material topics in the form of a written coursebook – shared as a PDF file. We then invite students to use NotebookLM (Google provided program). By uploading the teaching material to this online, free AI tool, the students are free to produce summaries, questions and answers and even podcasts of the course material. They can also just simply ask the AI questions about the course material. Only course material will be used by NotebookLM in this application, limiting the sources of error and discouraging the students from using whatever material they find themselves on the internet. The availability of such tools also helps the university comply with the requirement for universal design courses.

PE009

The 3Rs Grant Scheme at UNSW Sydney: Support for Development of Novel Alternatives

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Abstract
The University of New South Wales (UNSW) is one of Australians leading academic institutions, with a focus on research in the fields of biotechnology, engineering and medical science. The university's commitment to innovation and responsible progress is evident in their support of novel technologies aiming to replace animal use in research with alternative models and to develop methodologies to reduce the impact of research on wildlife.

Launched in 2019 and funded by the Office of Pro Vice-Chancellor of Research Infrastructure, UNSW's 3Rs grant scheme is unique nationally in providing generous support for the development of new methodologies and innovations by funding future projects. The scheme allocates $250,000 AUD annually to support proposals from UNSW affiliated researchers which are specifically evaluated on their potential to advance the 3Rs, on scientific merit, expected impact and feasibility. Over the lifespan of the scheme $1.44 million has now been awarded to sixteen projects whose primary focus is developing the 3Rs in Australia. Funded projects have included development of an open-source virtual nervous system to enhance testing of medical devices, refinement of animal identification and tracking in wildlife studies, development of bioengineered organs[Davis *et al*] and 3D cell culture techniques such as organoids to replace a specific animal model.

This presentation will present the scheme and highlight some of the exciting initiatives that have been supported over the past five years.

PE010

ASSET International Project: Digital Tools for the Management and Sharing of Research Animal Samples

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Abstract
The ASSET (Animal Sample Sharing & Efficient Tracking) project is an international pilot initiative that aims to promote an efficient use of experimental research animals and their tissues as well as stimulating innovative research ideas through the establishment of a searchable database for sharing biological samples.

ASSET seeks to popularize the acquisition of fresh animal tissue, maximizing the knowledge gained from each individual animal, and enabling the performance of pilot experiments and complementary studies on tissue samples from various

genetically modified animals with the final objective of reducing the number of animals used in research.

The ASSET project initially involves three institutions: the Umeå University in Sweden, PORT- Łukasiewicz Research Network in Poland, CREBA-IRBLleida in Spain together with the Spanish company NorayBio. The participants have already implemented and are currently using in production a software platform tested for over 20 years in human biobanking, now used for managing and tracing tissues obtained from research animals and publishing these in a catalogue network, available at the request of the scientific community.

ASSET organizes, systematizes and supports the large-scale storage of biological animal material with appropriate software tools. It enables professionalization, traceability of specimens and quick and reliable access to them. It introduces a reorganization of sample management that will become consistent across many institutes, prompting the appearance of future institutional biobanks of animal tissues and therefore, promoting a more efficient and ethical use of animals in research.

PE011

Influence of Genetic Background, Diet, Sex and Age on Digestibility in Polygenic Mouse Models

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Abstract
Digestibility studies in mice provide valuable insights into how genetic background and diet affect nutrient metabolism and energy balance. These factors are crucial for understanding metabolic disorders and for tailoring interventions. In our study, we investigated the effects of genetics, diet, age and sex on diet and water intake, fecal excretion, weight gain and apparent digestibility of crude protein and crude fat in two polygenic mouse models: the Fat (FLI) and Lean (FHI) lines. Mice were fed a high-fat diet (HFD) or a low-fat diet (LFD), and parameters were measured at prepubertal (6 weeks old) and adult (13 weeks old) stages.

Lean line digested HFD differently than the Fat line, with the differences being most pronounced in males. Compared to the Fat line, Lean line on HFD showed increased diet and water intake and weight gain in adulthood, followed by a decrease in fecal excretion. On the other hand, the Fat line showed the highest weight gain at prepubertal age and also an increased amount of feces excreted on a LFD regardless of age. Apparent crude protein digestibility was most efficient in Lean males on HFD, and apparent crude fat digestibility was higher in both lines and sexes on HFD. Digestibility was not affected by the age of mice.

These results highlight the complex interplay of genetics, diet, and age and reveal important differences in energy metabolism and nutrient utilization between the Fat and Lean lines, providing a basis for targeted strategies to improve metabolic health.

PE012

Behavioral Phenotyping of Unique Fat and Lean Mice to Explore Metabolism Impact on Behavior

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Abstract
Obesity results from an imbalance in energy metabolism, with skeletal muscles and the brain playing key regulatory roles. We study a unique polygenic mouse model for obesity (Fat) and leanness (Lean), developed through 60 generations of bidirectional selection. Fat mice have five times more fat mass than Lean mice, with no significant difference in food intake, and develop metabolic syndrome and diabetes. Our previous genetic mapping identified *Pla2g4e* as one of the most differentially expressed genes, whose product, cPLA2ε, is associated with energy balance and lipid metabolism. *Pla2g4e* overexpression in the hypothalamus and skeletal muscle of Fat mice suggests a potential behavioral impact.

We behaviorally phenotyped both lines at 3 and 8 months of age using the open field, elevated plus maze, three-chamber sociability test, light-dark box, and sucrose preference test. Fat mice were more exploratory, more active, and exhibited higher anxiety than Lean mice. No differences were found in anhedonia, but Fat mice consumed less liquid than Lean mice. Social behavior was sex-specific, with Fat females seeking more social interaction and showing better social memory than Lean females. Notably, age-related differences were most pronounced in the sociability index and social novelty preference.

These results suggest that *Pla2g4e*-associated metabolic perturbations may influence behavior across both sexes and age groups. Future studies using home-cage monitoring technology are needed for continuous and detailed behavioral and metabolic assessments, contributing to a broader understanding of the complex interplay between metabolism, behavior, and aging.

PE013

3D Printing in Laboratory Animal Science: Where 3D meets the 3Rs

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Abstract
3D printing has transformed biomedical research, yet its potential to drive the Reduction and Refinement of animal procedures remains underutilized within the laboratory animal science community. While bio-printing holds promise for replacing animal models in the future, conventional 3D printing technology already offers impactful solutions to enhance animal welfare and experimental outcomes in the present.

Despite the widespread availability of 3D printing in academic and research institutions, misconceptions about the complexity of design processes and the cost of equipment often deter its adoption. However, modern consumer-grade 3D printers provide an

affordable, user-friendly platform with virtually limitless applications in laboratory animal programs. Even with minimal experience in 3D design, researchers and staff can create fully customizable tools that refine a wide range of procedures, improve precision, and optimize training resources. This presentation highlights how a laboratory animal care program has successfully embraced 3D printing as an institution-wide initiative. Through the development and deployment of tailored 3D-printed solutions, the program has advanced the refinement of laboratory animal procedures and established innovative training tools. By showcasing practical applications and the tangible benefits of 3D printing, this work aims to inspire broader adoption of this accessible technology across the field.

PE014

Multicenter Validation Study of a 3D Printed Mouse Model for Surgical Training

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Abstract
A novel 3D-printed rodent surgical simulator was developed at the University of Arizona (UA) to serve as a reliable, animal-free platform for rodent surgical training. Following months of internal use at UA, a multicenter international validation study was initiated involving five research and academic institutions across Europe and the United States, aimed to assess the simulator's content validity (effectiveness as a teaching tool) and face validity (realism and appropriateness). A systematic approach was adopted to standardize testing and data collection across all participating institutions, ensuring objective and reliable results. Experienced and novice rodent surgeons were provided with standardized orientation materials and performed identical tasks using the same tools and supplies, with a pre-determined number of iterations. Participants completed a user feedback survey upon task completion, while standardized photographs and scoring criteria were used for performance evaluation in a double-blind manner across institutions. The content validity results highlight that inexperienced trainees demonstrated improved performance with repeated practice on the simulator, while the face validity findings indicate a high level of realism. Additionally, the participant pool expressed strong support for incorporating simulation-based training strategies in the laboratory animal field. These collaborative efforts among laboratory animal professionals from diverse institutions and regions highlight the importance of validating training tools and harmonizing training methodologies to advance research and improve animal welfare globally.

PE015

Microsurgical Technique Training: Pioneering Interactive Training Systems and Courses for Laboratory Animals

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Abstract
Rodent surgery, especially in mice, presents significant challenges due to their small size and limited visibility. This often leads to unnecessary tissue damage and variability in disease models. To address these issues, the National Laboratory Animal Center (NLAC) in Taiwan has developed an innovative microsurgery training system and courses in collaboration with UC Davis to enhance animal welfare and improve the reproducibility of rodent surgical experiments.

This cutting-edge interactive system enables real-time, bi-directional instruction, allowing instructors to monitor and annotate up to 12 trainees' microscope views on an 85-inch digital whiteboard. Beyond traditional demonstrations, instructors can display specific trainees' microscope feeds, highlighting key insights for the group. Immediate feedback on early-stage techniques serves as valuable teaching moments, significantly enhancing training efficiency and accelerating the learning curve for participants. The system incorporates multi-view technology, digital whiteboards, PTZ cameras, computers, and video switchers, allowing participants to simultaneously observe surgical setups and detailed procedures. The system also supports remote teaching, enabling interaction between instructors and trainees regardless of location. Additionally, it facilitates training in advanced techniques, such as embryo transfer and intravenous injections, offering a flexible, versatile, and efficient learning environment. All sessions can be recorded for future reference, enhancing long-term training benefits. Moreover, addressing the scarcity of mouse blood vessel-specific simulators, NLAC has also developed imitation modules for vascular ligation and catheterization, allowing trainees to practice essential skills before working on live animals. Integrating the new teaching system with simulator practice significantly decreases the use of animals while enhancing procedural success rates.

PE016

Activation Behavior and Testing of Animal Platelets According to ISO 10993-4 Standards

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Abstract
The directive 2010/63/EU requires consistent implementation of the 3Rs concept in the scientific use of animals.

This includes the reduction of expected distress through adequate experimental refinement.

In veterinary practice and in the context of the scientific use of laboratory animals, medical devices are regularly used that come into contact with blood cells, which are the subject of numerous studies and veterinary diagnostics.

However, the vast majority of these medical devices are only tested for biocompatibility with human blood according to the ISO 10993 set.

In order to prevent these medical devices from causing clinical complications, masking parameters of diagnostic interest and influencing study results by activating blood coagulation, they should first be tested for their species-specific hemocompatibility analog to the standards applying to medical devices used in humans.

Therefore we aim to examine the activation behavior of murine, ovine porcine and human platelets after exposure to platinum-cured silicone and borosilicate glass, materials that are used in numerous medical devices on a functional and molecular base via FACS and scRNA-seq.

Our studies highlight that there are differences in the activation behavior and biology between animal and human platelets.

These differences in the activation behavior of animal platelets have implications in the context of clinical practice and research for the choice of materials that come into contact with animal blood.

PE017

Opening the Doors: USARO's Vision for Strengthening Connections in Animal Research

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Abstract
The United States Animal Research Openness Initiative (USARO) is a coalition of dedicated individuals and organizations working to build trust and foster meaningful connections between research institutions and the public. USARO's mission is to support the responsible use of animals in research by encouraging the sharing of thoughtful, accessible information about the purpose, ethical oversight, and care that guide animal studies. By promoting clear communication, USARO aims to enhance public understanding of how animal research contributes to scientific and medical progress, while addressing misconceptions and concerns.

USARO highlights a range of practical strategies that institutions can adopt, such as publishing accessible summaries of research goals and outcomes, developing educational programs for diverse audiences, and fostering dialogue through open-house events and community partnerships. These initiatives demonstrate how research organizations can align their communication efforts with their unique goals and resources while reinforcing their commitment to accountability, scientific excellence, and ethical responsibility.

This presentation will discuss the broader impact of USARO's collective approach, emphasizing how fostering openness and engagement can strengthen public confidence and create more resilient, collaborative research environments. Attendees will gain practical insights into how institutions can design and implement initiatives that address stakeholder expectations, support constructive conversations, and highlight the critical role of research in advancing knowledge and improving lives. By encouraging collaboration and clear communication, USARO's work serves as a model for building sustainable connections that support both scientific discovery and a shared commitment to responsible research practices.

PE018

Refinements in Rodent Environmental Health Monitoring Methods

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Abstract
The Research Institute of the McGill University Health Centre, a hospital based Research Institute, has been using Environmental Health Monitoring (EHM) methods to replace the use of live rodent sentinels since 2018. Sampling of research animals (direct colony sampling) as well as sampling of dust and debris (ventilated rack exhaust air dust testing and equipment swabbing) has proven efficient to maintain the health status of multiple rodent colonies such as mice, rats, gerbils, hamsters and guinea pigs. These EHM methods have also been used to test and validate decontamination procedures of housing rooms, surgery rooms, experimental equipment and cage wash areas. To address housing placement constraints, our EHM program has been crucial in monitoring and maintaining the strict clean health status of an immunosuppressed mice colony (NSGTM) that has been housed and bred for 3 years in the same room with colonies having a different health status (MNV, *Rodentibacter* spp., *Helicobacter* spp., *Tritrichomonas muris*). As part of our program, we are also trying to validate novel decontamination techniques and we had the opportunity to test a UVD robot®, an automated equipment using UV-C light designed for decontamination of clinical spaces. We used the UVD robot to successfully decontaminate a decommissioned room with equipment that had previously tested positive for rodent pathogens, even after vaporized hydrogen peroxide decontamination. Our results show that a robust sentinel free program can be built and be continuously improved. We will also be covering recent updates from the 3Rs Collaborative (3RsC) on this topic.

PE019

The Australian and New Zealand Laboratory Animal Industry – Advocacy for a Better Future

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Abstract
The laboratory animal industry in Australia and New Zealand is primarily represented by the Australian and New Zealand Laboratory Animal Association (ANZLAA). Despite being isolated "down under", ANZLAA brings together professionals dedicated to the care, welfare, and ethical use of animals in research and teaching and consistently provides significant value for its members.

ANZLAA's members include a wide range of expertise, from daily animal care and veterinary support to ethics and regulatory management. The association promotes high standards of animal husbandry, health, and ethical conduct in research. It also encourages the development of alternative techniques to reduce the use of animals in research and teaching.

To name a few initiatives, ANZLAA has multiple special interest groups, a well-being committee, mentoring program, a popular annual conference that includes high-quality international speakers as well as talks on best practice and innovation.

Additionally, a successful "Animal Technician Week" is held in November.

We are signatories to the Openness Agreements on Animal Research and Teaching that were launched in Australia and New Zealand and we work closely with Understanding Animal Research Oceania. All this will an ANZLAA membership of around 1000 people.

Although ANZLAA provides excellent quality programmes and has a positive relationship with other laboratory science organisations (eg. AALAS), the Executive Committee believes that there is mutual benefit to working more closely together with FELASA organisations.

This presentation will provide an overview of how the Australia and New Zealand Laboratory Animal Association (ANZLAA) supports and provides advocacy for our community and members.

PE020

MorphoPHEN: a Joint European Master Degree on Human Diseases Models Morphological Phenotyping

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Abstract
The scientific community currently lacks knowledge and expert human resources in mouse anatomy and pathology at a time when morphological phenotyping of the mouse as a model for discovery and mechanistic research, purpose-driven in vivo translational studies, and preclinical validation for human therapeutics is increasing. To address this situation, the European Commission recently funded the European Joint Master degree in "Human Diseases Models Morphological Phenotyping" (MorphoPHEN) (https://morphophen.eu). MorphoPHEN includes training in anatomy, imaging technologies, pathology, and machine learning as it applies to automated image analysis. Objective is to develop and implement a novel European and global training programme on mouse morphological phenotyping that increases capacity for robust, reproducible, and truly comparative analysis and understanding of data generated using the mouse as a model. This master combines the excellence and expertise in mouse anatomy, imaging, pathology, and machine learning at four institutions: UAB, UNINA, Aristotle University of Thessaloniki, and the Veterinary School at Lisbon.

The MorphoPHEN consortium is supported by a multidisciplinary network of associated partners by giving lectures, hosting students to carry out the Master thesis and participating in the scientific boards of the master's programme. Two Universities, the University of Cambridge and the University of Copenhagen support MorphoPHEN. Furthermore, five major European and North American mouse clinics equipped with integrated facilities for production and characterization of genetically engineered mouse models are also associated, along with CHARLES RIVER, the veterinary pathology core at St Jude Children's Research Hospital, and a company leader in the field of preclinical imaging.

PE021

Sex as Biological Variable: How to Help Researchers to Implement It in Animal Assays?

V. Dangles-Marie[1] and K. Ancelin[1]
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Abstract
Sex and gender are well known to have an impact on many physiological and pathological conditions. These features are more and more taken into account in clinical studies, while this is still poorly the case in animal research.

Within the Animal Ethics Committee of our institute, we have observed this shortcoming. We report here on the measures we have taken to change practices. We have largely based our work on N. Karp's approach [1]. In this presentation, we will illustrate the strategies chosen to i) convince researchers to integrate this variable into their experimental design and ii) to help them for analysis of the resulting data.

PE023

Treadmill Habituation Technique for Yucatan Minipigs in Neurological Studies

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Abstract
Yucatan mini pigs are commonly used as models in neurological studies due to their similarities to humans. In this context, the evaluation of gait changes is a key parameter. However, data on mini-pig gait kinematics remain limited, and assessment methods are often subjective.

The primary objective of this study is to refine a treadmill walking training protocol to minimize animal stress, improve their adaptation to the equipment, and thus ensure reliable kinematic data.

Training sessions were conducted on a single animal using positive reinforcement techniques. These included the use of a clicker and food rewards, to facilitate learning and reduce stress. The training was divided into three steps:

- Habituation Phase: association of the clicker with the reward, learning to follow a target.
- Static Phase: Habituation of the pigs to the stationary treadmill, involving reward sessions and gradual exposure.
- Dynamic Phase: Transition to using the moving treadmill, with a progressive increase in duration based on the animals' comfort and response.

After 28 days of training, the receptive animal demonstrated significant cooperation, accompanied by a noticeable reduction in behavioural stress signs.

The initial data obtained through infrared cameras proved sufficiently accurate to assess gait kinematics. These results demonstrate that this protocol allows for reliable locomotion data collection while minimizing animal stress. This approach contributes to improving the quality and relevance of neurological studies involving porcine models.

PE024

Diversity Outbred mice - A Model Organism with Genetic Diversity and its Own Challenges

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Abstract
Humans are genetically diverse, whereas conventional laboratory mouse strains consist of mice that are genetically uniform. Biomedical development and basic research in a single inbred strain is equivalent to studying and treating a single patient. All people are different, so understanding how treatments, interventions and diseases arise in the context of genetic diversity requires an animal model with genetically diverse individuals.

Jackson Laboratory introduced the Diversity Outbred Population for this purpose. Based on 8 founder strains, a breeding program was established in which each animal represents a unique combination of the founder genomes. There is a high degree of heterozygosity due to the increasing number of recombinations with each generation. The genome sequence of the founders is known and allows the reconstruction of the assembled genomes based on genotyping arrays.

The animals are not only genetically but also physiologically unique. Phenotypes are also created that go beyond the repertoire of the founder. Due to the wild strains, some individuals also exhibit very nervous or hyperactive behavior. This means that no live animals are released for transportation by air. It was therefore necessary to set up the institute's own breeding program. For this purpose, different cryopreserved DO lines were imported and these were prepared, reared and continued over several generation using a special breeding scheme. In our institute, the animals are used, e.g., to understand the genetic basis of infectious diseases. In particular, we are focusing on creating a comprehensive genetic atlas of the disease determinants of typhoid fever.

PE025

Development of Motorized and Automated Micromanipulation Systems for Reproductive Engineering

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Abstract
Micromanipulation of embryos and gametes of laboratory animals is used for gene modification, genetic testing, preservation of strains and genetic resources, and production of offspring. Those techniques are performed using a micromanipulator, but long-term training is required to ensure the reproducibility of micromanipulation. Therefore, we developed an auto-micromanipulation system (AMMS) by motorizing and automating the micromanipulator, with the aim of enabling reproducible operation by anyone after simple training[1]). In this report, we introduce the application of the AMMS in two reproductive engineering techniques. Intracytoplasmic sperm injection (ICSI) is useful for producing offspring as it enables the fertilization of sperm that have become immobile due to cryopreservation or disease. Blastomere biopsy (BB) is used for preimplantation testing of background genes and introduced genes. The AMMS performs these techniques by semi-automating the pipette movement (three-dimensional), sample positioning (two-dimensional), injector aspiration and discharge, and microscope field focusing. Gametes and embryos used in this study were obtained from sexually mature B6D2F1 mice. In ICSI, more than 80% of the sperm injected oocytes were fertilized, and the fetal development rate was equivalent to that of embryos derived from in vitro fertilization. In BB, all biopsy-treated 8-cell embryos survived, and the fetal development rate was equivalent to that of the non biopsy group. These results suggest that the AMMS can be used even by beginners to successfully perform minimally invasive and reproducible ICSI and BB.

PE026

Verification and Validation of *in Vivo* Translational Digital Biomarkers to Advance Preclinical Animal Testing

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Abstract
Emerging technologies that provide continuous monitoring of animals in their home cages offer immense opportunities to improve research animal welfare, scientific data quality, and translation. They offer key advantages over traditional methods of measuring animal behavior and physiology that are limited by infrequent sampling and stressful environments, potentially compromising wellbeing and research outcomes. However, the adoption of these technologies is not yet widespread due to a number of barriers.

Therefore, to assist in advancing these impactful technologies, the 3Rs Collaborative's Translational Digital Biomarkers (TDB) initiative has united stakeholders through shared experiences and thought leadership. Furthermore, to ensure the reliability and relevance of digital biomarkers, they have adopted a variation of the Digital Medicine Society's (DiMe) V3 Validation Framework for preclinical contexts. This approach emphasizes verification, analytical validation, and clinical validation to ensure the reliability, precision, and applicability of digital biomarkers. We will present key considerations for engaging in digital biomarkers and the approach for their verification.

Ultimately, by fostering interdisciplinary collaboration and integrating robust validation processes, we can accelerate the adoption of new techniques, ultimately advancing translational research and ensuring research animals benefit from technological evolution.

PE027

Reproductive Sciences: Supporting the Animal Experimentation of Tomorrow

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Abstract
The advancement of techniques in creating research models has led to the development of increasingly relevant models to meet the needs of the scientific community. This progress has also resulted in a significant rise in the number of genetically modified animals available for research. In this evolving landscape, Assisted Reproductive Technologies (ARTs) have become indispensable for the effective management and utilization of mice models.

Techniques such as superovulation, in vitro fertilization (IVF), embryo transfer, and cryopreservation are pivotal. Superovulation boosts the yield of viable oocytes, facilitating the production of more embryos through IVF. Embryo transfer supports the propagation of genetically modified lines, while cryopreservation ensures long-term genetic material preservation.

These methods offer a superior alternative to traditional breeding, enabling quick access to experimental animals, accelerating research timelines, and improving production efficiency and animal quality. They also address logistical and ethical challenges in large-scale breeding.

Janvier Labs' comparative studies highlight the advantages of these tools over standard breeding, emphasizing more ethical animal sourcing, better adherence to the 3Rs (Replacement, Reduction, and Refinement), and significant cost savings, promoting a more sustainable approach to animal model production.

PE028

Addressing Aortic Aneurysm Disease in Human-like LDLR-/- Minipigs: From Animal Models to Clinical Applications

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Abstract
In case of rupture, abdominal aortic aneurysms (AAAs) are often a death sentence for the patient, finding new ways of preventing this event is of great significance. Currently, all available treatment options require surgery, which can place significant strain on patients. In our study, we triggered infrarenal AAAs in low-density lipoprotein receptor knockout (LDRL-/-) Yucatan minipigs via porcine pancreatic elastase perfusion (PPE-model) in combination with collagenase and CaCl2. Those animals develop hypercholesterolemia and human-like atherosclerotic lesions when fed with a high-fat diet, which are both risk factors for developing AAAs. We intervened 7±1 days into the trial by inflating a drug-eluting balloon (DEB) coated with anti-miR29b antisense oligonucleotides (ASOs). It is known that the inhibition of miR29b using ASOs will increase the abundance of collagen in the vessel wall and therefore stabilize the aneurysm by inducing fibrosis. To evaluate the success of our treatment, we measured the aortic diameter using ultrasound. The results demonstrated that this procedure can reduce aneurysm growth compared to the control group, which received only a sham-coated DEB. There are no signs of a systemic reaction in the bloodwork and the histochemical analysis of the aneurysmal sack and other tissues of interest looks promising. By working in this highly translational model of the aortic aneurysm disease, we aim to gain insights into how human patients will respond to this new minimally invasive treatment.

PE029

Rehoming Laboratory Beagles from Pharmaceutical Company – Part of Organizational Responsibility in CoC

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Abstract
Experimental animals and their welfare pose an ethical dilemma and public concern. Legislation and public opinion support rehoming instead of euthanasia for healthy laboratory animals after they are no longer suitable for experimental use. Directive 2010/63/EU gives an opportunity to rehome laboratory dogs after they are no longer needed for experimental use. Euthanasia at the end of animal's experimental use – if the research does not require it - is unnecessary and possibly against the objectives of European directives.

Rehoming offers laboratory animals a good quality of life in retirement, which compensates their restricted experiences while being kept in the laboratory environment and undergoing procedures. Successful adoption and transition to a family pet lifestyle relieves the emotional experiences that such animals have had during their life.

The knowledge that animals will be rehomed instead of killed is reducing work-related stress of researchers, veterinarians and animal caretakers. It also validates their attachment to the animals in their care. Having a program for rehoming animals is also an initiative-taking way to help staff cope and is a part of organizational responsibility in Culture of Care. Personnel who cannot cope with the loss of animal life that is inherent in most animal research programs may need professional support.

Orion successfully piloted rehoming with two Beagle dogs in autumn 2022, and in December 2024 new loving homes have been found already to 18 dogs. Many people participate in rehoming process - designated veterinarian, animal caretakers, researchers, staff from animal protection organization and new homes.

PE030

Bridging Leadership Gap: Manchester's Novel Postgraduate Programme in Biomedical Research Facilities Leadership and Management

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Abstract
The increasing complexity of biomedical research facilities demands leaders who can balance scientific excellence with operational efficiency, ethical considerations, and financial sustainability. Despite this growing need, there remains a significant gap in specialized education programmes that address the unique challenges of managing these sophisticated research environments.

We present the University of Manchester's innovative Postgraduate Certificate in Biomedical Research Facilities Leadership and Management, launching in 2026. This programme, developed in collaboration with leading international institutions and AAALAC International, offers a comprehensive curriculum focusing on three critical areas: Facility Management and Quality Assurance; Ethics and Animal Welfare; and Budgeting and Finance.

The flexible, primarily online format with optional in-person workshops makes it accessible to working professionals globally. Distinguished faculty from renowned institutions, including the Universities of Copenhagen, Leiden, Oxford, and Yale, ensure world-class education delivery. The programme emphasizes practical application through case studies, virtual discussions, and hands-on workshops, preparing leaders to navigate complex

regulatory requirements while maintaining the highest standards of animal welfare.

Future development plans include additional specialized units and a dissertation component, creating pathways to both a standalone Master's degree and Manchester University's Global MBA programme. This pioneering initiative represents a significant step forward in professionalizing research facility management and establishing global standards for excellence in laboratory animal science leadership.

PE031

Development of Training Simulator for Laboratory Animal Experiment

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Abstract
Background: Training of hands-on techniques for laboratory animal experiments using live mice and rats must adhere to the 3Rs principles (Refinement, Reduction, Replacement). With increasing animal usage in education in Korea, alternatives are needed to reduce animal numbers and minimize their distress. To address this, rat and mouse simulators have been developed to ensure anatomical accuracy and to support various training programs in animal experimentation.

Materials & Methods: Computed tomography scans of mice and rats were performed under the approval of the Institutional Animal Care and Use Committee (CUMS-2024-0150-01). The three-dimensional images were converted into stereolithography format, and molds were manufactured using acrylonitrile butadiene styrene resin through fused deposition modeling. Silicone materials of varying hardness were used to fabricate organs, skin, and other structures for durability and tactile realism. The oral administration site was constructed with resin to improve detail.

Results: Two simulator models were developed. The basic training model of mice and rats supports fundamental skills such as handling, restraint, drug administration (oral, intraperitoneal, subcutaneous, intravenous, intramuscular), and ear punching. The dissection training model of rats focuses on the shape, location and structure of thoracic and abdominal organs, providing foundational education for surgical procedures. Both models aim to enhance the technical proficiency of novice researchers.

Conclusion: The simulators reduce animal use, allow for safe and repetitive practice, and minimize harm to animals, offering ethical, and efficient training alternatives. By supporting the 3Rs principles, these tools enhance educational outcomes and improve the quality of research in laboratory animal experiments.

PE032

Exploring the Role of Gut Microbiota in Microscopic Colitis: A Novel Murine Model

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Abstract
Collagenous colitis (CC) and lymphocytic colitis (LC), subtypes of microscopic colitis (MC), are associated with inconvenient symptoms that significantly reduce patients' quality of life. This study uses a novel murine model to explore gut microbiota's role in MC pathogenesis. We hypothesize that specific alterations in the intestinal microbiome play a crucial role in MC development, marked by the suppression of beneficial bacterial populations and the proliferation of potentially harmful species

Our research employs C57BL/6 mice (8 weeks old, n = 10/group) across seven treatment groups, utilizing antibiotic treatment, bowel cleansing, and fecal microbiota transplantation (FMT) from MC patients. Through 16S rRNA sequencing and blood analysis at multiple time points, we aim to identify microbial signatures associated with MC and correlate gut microbiota changes with post-FMT MC symptoms.

Results reveal significant microbial and host physiological alterations in the MC mouse model. Key findings include suppression of Helicobacter and Tyzzerella, alongside increased Parabacteroides across treatment groups. Immune cell analysis demonstrates a dynamic pattern, with white blood cell and lymphocyte counts initially declining, peaking at 8 weeks, and stabilizing by 24 weeks. Granulocytes exhibit significant fluctuations, peaking at 16 weeks, while monocyte levels remain stable.

This study establishes a temporal model for MC, highlighting distinct changes in microbiome and immune cell dynamics. Our findings provide crucial insights into the interplay between FMT and host-microbe interactions in MC pathogenesis, potentially informing novel diagnostic and therapeutic strategies for the disease.

PE033

Comparison of In Vivo and In Vitro Results of Medical Devices Sensitization Testing

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Abstract
Sensitization is an obligatory prerequisite of biocompatibility assessment of medical devices. The recently amended standard ISO 10993-10:2021 allows only in vivo test methods on animals or humans, but includes also a list of non-animal methods, validated for neat chemicals, which might be used after verification of applicability for complex mixtures as medical devices or their extracts. Our pilot study aimed to compare results of regulatory testing of 10 real life medical devices by Local Lymph Node Assay (OECD TG 442A) with results of a battery of assays covering all three key events of the Sensitization Adverse Outcome Pathway (DPRA, OECD TG 442C; LuSens, OECD TG 442D; h-CLAT, OECD 442E). The samples were extracted or diluted in saline solution, DMSO and/or culture medium. The 4 positive samples in the in vivo test were correctly classified by the battery of non-animal tests using "the 2 of 3" principle of the Defined Approaches for Skin Sensitisation (OECD TG 497). One sample (rubber glove) was negative in vivo and positive in vitro, showing the high sensitivity of the in vitro methods to latex and similar compounds. The presented results confirm the feasibility of non-animal methods for skin sensitization of medical devices and provide information on the optimization of these methods in accordance with the current European Commission initiative to define a Roadmap to phase out animal testing in the EU. *Supported by Ministry of Health, Czech Republic - conceptual development of research organisation („National Institute of Public Health - NIPH, IN: 75010330").*

PE034

The AK KAB Cage Reprocessing Working Group: Information about Latest Brochure 7th Edition 2024

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Abstract
The cage processing center is the heart of animal husbandry! A wide variety of machinery, accessories and logistics components are used to reprocess and supply the animal husbandry with hygienically clean and sterile cages and accessories.

To ensure trouble-free and economical operation, many important parameters such as the necessary capacities, the right choice of equipment and processes, ergonomics and employee protection, equipment redundancies, the necessary space requirements and logistics components must be taken into account as early as the planning phase.

For over 15 years, the AK KAB working group and its experts have been dealing with these issues and publishing manufacturer-independent recommendations in various publications.

The reprocessing of animal cage systems and accessories is both resource- and cost-intensive. Limited personnel resources and increasingly expensive supply media lead to a considerable increase in operating and reprocessing costs. The legally prescribed achievement of climate protection targets, which also significantly affects the highest possible sustainability of buildings (new construction, expansion or conversion) and the operation of research facilities, is a further challenge that also has an impact on the design of reprocessing centers, facilities and processes.

The aim of the presentation is to introduce the 7th edition of the AK KAB working group and its updated recommendations, which were published at the last GV-SOLAS conference in Wuerzburg 2024.

In particular, the new features of the brochure include recommendations for the particularly sustainable and automated design of plant and treatment processes, while at the same time ensuring testable and certifiable results.

PE035

Practical Solution for the Safe Handling of Tamoxifen in Laboratory Animal Facilities

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Abstract
Introduction: Tamoxifen is a selective estrogen receptor modulator commonly used in the treatment of breast cancer in humans. In animal research, tamoxifen is increasingly administered to induce tissue-specific gene expression in genetically modified animals. Due to its carcinogenic, teratogenic and mutagenic potential for humans, tamoxifen must be handled with caution.
Description: Laboratory animal care takers and researchers may be exposed to harmful substances when they administer hazardous chemicals such as carcinogens, reproductive toxins or other highly toxic substances to animals or handle cages or bedding of animals that excrete these substances or their toxic intermediates [1]. Increasingly, tamoxifen is being administered to genetically modified animals via feed or injection to induce tissue-specific gene expression. Tamoxifen and its two bioactive metabolites (hydroxytamoxifen and endoxifen) are temporarily excreted in the animals' feces and urine after administration, posing a short-term potential health risk to people handling these animals. We have therefore drawn up a practicable SOP for our animal facility in collaboration with animal care staff and experimenters in order to reduce the exposure potential for persons working in the animal facility as far as possible.
Conclusion: Targeted measures and structured work processes can significantly reduce the risk of exposure to harmful substances such as tamoxifen for animal husbandry staff.

PE036

IMP3ROVE Animal Research

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Abstract
The European Union-funded COST Action, "3Rs concepts to IMPROVE the quality of biomedical science" (IMPROVE) aims to establish an interdisciplinary network dedicated to refining, harmonising, and promoting the adoption of 3Rs concepts to enhance the quality of biomedical research1. Central to IMPROVE's mission is the conviction that further adoption of the 3Rs principles will inherently lead to better research practices and outcomes. This conviction is particularly pertinent in the context of the reproducibility crisis, where heightened awareness has sparked critical discourse within the research community regarding the reliability of data from animal studies. The 3Rs principles play a pivotal role fostering stakeholder engagement and promoting shared responsibility for ethical scientific conduct as an imperative.

IMPROVE's strategic focus revolves around capacity building and coordination across four key domains: research education, 3Rs implementation, dissemination, and research quality and translatability. Through the collaborative efforts of dedicated working groups, IMPROVE aims to implement these targeted activities. Additionally, an Ethics Crossover Group has been formed that focuses on supporting the discussions on the different ethical points of view that research with or without animals implies2.

IMPROVE's initiatives will be presented underscoring their potential to unite and strengthen the fragmented landscape of 3Rs practices. The network has as a goal to substantiate the notion that the 3Rs principles are intrinsically intertwined with the pursuit of good science. This can be achieved by engaging young scientists, established researchers, and stakeholders through workshops, training schools, and other activities.

This work is supported by the COST Action IMPROVE, CA21139.

PE037

Can We Replace Sentinels? Comparison of Sentinels to Animal Free Methods for Health Monitoring

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Abstract
The use of sentinel animals is currently the standard in routine health monitoring in mouse facilities. Even though there are infectious agents known to be missed by sentinels and that alternatives have been available for quite many years. There are various options within environmental monitoring, both with regards to type of collection material and how it is used. The goal of this study was to compare the efficacy of environmental monitoring with standard soiled bedding sentinel animals.

Two different environmental monitoring methods exhaust air dust (EAD) and sentinel-free soiled bedding (SFSB) and three different collection materials were evaluated and compared to sentinel animals in both a semi-experimental and standard facility set-up. The results obtained indicated a variation in the efficacy of different environmental testing approaches, with some being superior and some inferior to sentinel animals. The efficacy of the tested environmental monitoring strategies in detecting the presence of common infectious agents in colony animals will be presented and compared to using live sentinels.

PE038

Utilizing Common Marmoset (Callithrix jacchus) as Models for Diabetes Research

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Abstract
Murine models have significantly advanced the study of mechanisms and the development of therapies for diabetes mellitus. However, they exhibit physiological limitations when applied to the study of biopharmaceuticals and islet transplantation. Common marmosets are attractive models for such studies due to their physiological similarities to humans and their small size. In this study, we conducted two experiments to explore the utility of common marmosets in diabetes research. The first was a glucose tolerance test (GTT) using chair restraint in common marmosets. The fasting blood glucose level (16 hrs) of common marmosets averaged 100–120 mg/dL, returning to normal within 30 minutes following intravenous glucose administration and within 90 minutes following oral glucose administration. Next, a type 1 diabetes model was established through partial pancreatectomy in combination with streptozotocin (STZ) administration. After the second dose of STZ (50–100 mg/kg), diabetes was diagnosed based on sustained hyperglycemia lasting more than 3 days, defined as a non-fasting blood glucose level exceeding 250 mg/dL. Urine dipstick testing revealed glucosuria exceeding 1000 mg/dL in the diabetic marmosets. Other diabetes markers, such as hemoglobin A1c and glycated albumin, increased by 2-fold and 1.5-fold, respectively, compared to levels prior to diabetes induction. Histological examination of the diabetic models showed a marked reduction in pancreatic beta cells. These findings will contribute to the evaluation of the effectiveness and safety of innovative therapies, such as cell and antibody therapies, and to comparative studies of metabolic diseases and metabolomics.

PE039

Analyzing Strain-specific Ultrasonic Vocalizations in Mouse Pups on Postnatal Day Eight Using DeepSqueak

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Abstract
Ultrasonic vocalizations (USVs) emitted by rodent pups are critical for mediating maternal behaviors, serving as primary communication signals to facilitate mother-infant bonding and ensure pup survival. These vocalizations elicit robust maternal retrieval responses and undergo developmental changes during early postnatal life, with call frequency peaking around postnatal day 8 (Caruso et al., 2022). Advances in machine learning, such as the DeepSqueak platform (Coffey et al., 2019), offer powerful tools for the automated detection and classification of USVs, enabling large-scale analyses of these behaviors.

Using DeepSqueak, we analyzed USVs in two inbred mouse strains [C57BL/6J, BALB/cByJ]. We recorded isolation-induced USVs on postpartum day 8 for five minutes in 191 pups (C57BL/6J males: 48, females: 47; BALB/cByJ males: 50, females: 46). Totally, 34'626 calls were detected and classified into 17 call clusters via unsupervised clustering provided by the DeepSqueak software. One cluster was identified as noise and was excluded for further analysis, resulting in 16 distinct call clusters. Strain-specific differences were observed in key acoustic parameters, with pups from the BALBc/ByJ strain vocalizing lower frequencies, emitting longer and louder calls compared to C57BL/6J pups. Additionally, the number of calls per cluster and transitions between clusters differed between the two strains.

These findings underscore that strain-specific differences in USVs are already apparent in the early postnatal period, potentially reflecting divergent communication for maternal interaction and pup survival. While advanced tools like DeepSqueak enable the detection of nuanced patterns, the behavioral consequences of these acoustic differences remain unclear and require further research.

PE040

Education in Laboratory Animal Science Using the Adaptable Online Platform LAS Interactive

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Abstract
LAS interactive is a tri-lingual online platform (German, English, French) for laboratory animal science, consisting of "vtk online" (German "Versuchstierkunde online"), a free information portal that covers common laboratory animal species as well as other species such as spiny mice, dwarf hamsters, bats and others; while "las campus" offers online training to attain theoretical knowledge to work with laboratory animals according to EU recommendations or individual modules e.g. on national legislation or species-specific content.

One of the main features is the collaborative approach: Content can be adapted to country specific or institutional requirements and thus be supplemented with information relevant for a specific country or at an institution level. This content can be added/updated at all times without the need to redesign a course or module. Quizzes and online exams help focus and demonstrate achieved study objectives. The current questions database consists of well over 1000 questions in all three languages, addressing the different modules and learning outcomes. Each study question cites the corresponding content of las campus and each correct answer is linked to the respective study content of the course or module. Exams can be set up for groups or individuals, scheduled at specific times or be available for a certain time period.

The platform was originally developed in 2005 as a study aid for students of the life sciences but since than has been established as an online tool that is widely used today.

PE041

Facilitating and Encouraging Preregistration

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Abstract
Preregistration of experiment protocols contributes greatly to scientific quality, e.g. by making it possible to compare published results with the original study design. To facilitate preregistration of animal studies, our institute has set up a collaboration with PreclinicalTrials.eu in which researchers can easily upload their experiment protocols to an online accessible database. Since the start of this collaboration in November 2023, the Animal Welfare Body (AWB) in Nijmegen has been encouraging researchers to upload their protocols before the start of an experiment. As a result, the number of preregistrations at our institute has been rising steadily. Here we will share how we informed and motivated researchers, and which questions and challenges we encountered. First, researchers were informed through a newsletter and institute meetings. Next, every time a new protocol was discussed between researcher and AWB, a standard question was asked on whether the protocol would be uploaded to the database, and if not, what the reason was to decline. Unfamiliarity with the procedure was a first challenge, together with confusion from researchers in basic science areas on whether their work belonged in a database with Preclinical in the name. These concerns could largely be addressed by providing more explanation. The AWB encouraged discussions on preregistration within research departments and also approached PI's directly, which led to the commitment of several research groups to preregister their future protocols. Concerns about intellectual property often could be alleviated by submitting under an embargo. Together, these actions contribute to making preregistration standard practice.

PE042

Pig Model for an Aphersis Procedure: A Proof-of-Concept Study

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Abstract
Therapeutic Plasma Exchange (TPE) with an eggshell membrane-based apheresis column was evaluated for safety and feasibility in a porcine post-chemotherapy model. The column was designed to adsorb platinum compounds, such as Oxaliplatin, during TPE.

A total of 6 pigs were used, selected for compatibility with the apheresis machine and required exchange volume. Two groups were studied: one to validate the procedure and the other to assess the column biocompatibility. Each session, including chemotherapy, a 30-minute gap for oxaliplatin's action, and apheresis, took approximately 4 hours. The procedure was completed when the animal plasma volume had been processed through the eggshell membrane. Mechanical ventilation ensured proper anesthesia and recovery. A one-week rest period between sessions allowed the animals to recover.

Hematological and biochemical parameters were monitored, revealing no major abnormalities between the groups; observed changes were transient, with full recovery over time. Leukocyte levels decreased and calcium levels increased after each TPE but returned to baseline values at the start of subsequent procedures. Improvements in the anesthesia protocol, using a combination of different maintenance agents, led to better recovery in the animals. The apheresis column demonstrated biocompatibility, suggesting it could enhance platinum removal during TPE, potentially improving chemotherapy outcomes without significant adverse effects.

This approach could provide a novel and sustainable method to improve chemotherapy outcomes and reduce associated side effects, paving the way for future clinical applications. The porcine model was crucial in demonstrating the feasibility and safety of this procedure, highlighting its relevance in preclinical studies for therapeutic apheresis.

PE043

Replacement Models Using Intestinal Organoids to Study Tuft Cell Differentiation and Activation

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Abstract
Organoids are three-dimensional in vitro tissue models replicating morphology and functionality of in vivo tissues, reducing dependence on animals. Among their applications, organoids provide a powerful tool for investigating intestinal physiology. Tuft Cells, a rare population of chemosensory sentinel cells in the intestinal epithelium, respond to luminal pathogenic stimuli and play a critical role in immune responses. Despite their significance, mechanisms regulating Tuft Cell activation remain unclear. A defining characteristic of Tuft Cells is the expression of choline acetyltransferase (ChAT), enabling acetylcholine release, a feature otherwise restricted to cholinergic neurons.

This study investigated Tuft Cell differentiation and activation using intestinal organoids from ChAT-Cre/Ribotag mice, which express a fluorescent marker in ChAT+ cells. Organoids were cultured in basal ENR medium (Epidermal Growth Factor, Noggin, R-Spondin) or media with interleukin-4 (IL-4) or interleukin-13 (IL-13). Analyses included qPCR of stimulated organoids to assess gene expression changes. Additionally, single-cell RNA sequencing data from an open-access dataset (Nature, 634:929–935, 2024; Huang et al.) complemented findings on Tuft Cell differentiation and gene expression.

In basal conditions, no fluorescent signal was observed, indicating the absence of ChAT+ Cells. However, IL-4 and IL-13 exposure significantly induced differentiation of ChAT+ cells, confirmed by fluorescence imaging. These findings suggest IL-4 and IL-13 drive Tuft Cell differentiation in organoids.

This study underscores the utility of intestinal organoids, as a robust, ethical model for exploring Tuft Cell biology and immune mechanisms. Intestinal organoids offer valuable insights into Tuft Cell function and represent a promising alternative to animal models in biomedical research.

PE044

Paving the Way Forward: Learning from Past 3Rs Funding Success to Shape Future Opportunities

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Abstract
The Swiss 3R Competence Centre's Open Call funding scheme (2018–2020) attracted 223 applications from 25 different institutions across Switzerland, demonstrating widespread interest in advancing the 3Rs principles. We analysed the applicant demographics, scientific fields, review and selection process for all applications we received. Of these applications, the majority focused on Replacement (41%), followed by combined Replacement/Reduction projects (17%), and pure Reduction projects (17%). The most represented research areas were nervous system and cancer research, aligning with areas where animal experimentation is most prevalent. With a total budget of CHF 3.5 million, 15 high-quality projects were funded, led by 9 men and 6 women from 8 different institutions. The funded projects showed diverse approaches: 6 focused on implementation of established methods, 5 on method development, 3 on basic research, and 1 on pilot studies. By 2024, these projects had generated 33 peer-reviewed publications, with more expected as projects reach completion.

This comprehensive analysis, and follow-up report from our Scientific Advisory Board provide valuable insights for shaping future funding strategies. The strong interest in Replacement methods, particularly in key research areas, suggests a positive

shift toward alternative methods. Moving forward, these patterns can inform targeted funding approaches, help identify gaps in 3Rs coverage, and optimize resource allocation. By continuously monitoring such trends and adapting funding schemes accordingly, we can better support the scientific community's transition toward more ethical and efficient research practices while maximizing the impact of available resources."

PE045

Using the TRL Scale to Manage the Development of Alternative Technologies to Animal Biomodels

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Abstract
Scientific research in health has historically relied on animal biomodels to achieve therapeutic advancements, significantly improving human health by enabling the study of diseases, drug testing, and treatment development. However, ethical concerns and the challenges of translating animal-based findings to humans have spurred the search for more accurate and humane alternatives. Aligning with the principle of the 3Rs (replace, reduce, refine), the development of innovative methods to minimize animal experimentation has become a global priority.

This study proposes the application of the Technology Readiness Level (TRL) scale as a management tool to evaluate and promote the development of alternative technologies. Originally used in other fields, the TRL scale was adapted to health research, providing a structured framework to assess technological maturity.

The research aimed to create an Information System (IS) incorporating the adapted TRL scale to monitor and manage the progress of alternative methods. The methodology included a literature review to identify alternative methods, analysis of TRL frameworks, definition of tailored criteria, and development of an IS prototype. This led to the creation of the Alternative Methods Readiness Level (AMRL) system, enabling the registration, evaluation, and tracking of projects.

By centralizing data and offering a systematic approach to assess technological progress, the AMRL system fosters ethical, sustainable practices and optimizes resource allocation. The prototype demonstrated potential for validating humane and precise methodologies, reducing reliance on animal models, and advancing global trends in ethical research.

PE046

How to Calculate the Carbon Footprint of an Animal Facility

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Abstract
Calculating the carbon footprint requires quantifying the amount of greenhouse gases emitted annually in relation to specific activities. As a case study, we selected the "Mice Animal Research Unit," where we will apply the methodology outlined in the Greenhouse Gas (GHG) Protocol, established in 1997 by the United Nations in Kyoto. The GHG emissions of buildings are calculated based on energy consumption for heating, electricity, and refrigeration. Emissions from procurement are estimated using a monetary factor, where €1 spent corresponds to 917 kg of CO_2 equivalent (eCO_2). GHG emissions from business travel are calculated based on the modes of transportation used, including cars, public transport, and planes. To effectively reduce CO_2 emissions, the first step is to measure current emissions and then identify strategies to minimize them. This involves collecting laboratory data in accordance with the carbon accounting categories defined in the Kyoto Protocol. The emission factors identified in the ADEME (France) database are then used to calculate the laboratory's carbon footprint and lead initiatives to reduce the carbon footprint. The results show that purchases represent around 60% of greenhouse emissions, that agents' travel to work is in 2nd position with more than 15% and that energy is in 3rd position with almost 12% of emissions. Many uncertainties remain but effective solutions are possible.

PE047

Embedding a 3Rs Strategy into a Training Framework

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Abstract
The 3Rs are an important but often included as a standalone training module, which may lead to staff feeling that it is an added extra rather than an integral part of the day job. Many refinements are often included without being more broadly highlighted because *'it is just what we do to improve animal welfare, and it is too small to share with others'*, and this can be the case for some reductions and replacements. Promoting the 3Rs across a training framework is imperative for several reasons: (1) highlighting how we want staff to integrated and consider the 3Rs, (2) ensuring staff understand the differences between each R and when it should be considered during the life of a programme of work. (3) The importance

of promoting and discussing successes both internally and, where appropriate, externally to share good practice in 3Rs. (4) support PPL holders in their 3Rs commitments and reporting.

This presentation will outline some of the barriers to promoting and considering the 3Rs and how these may be mitigated, through the inclusion of the 3Rs strategy across the training framework aiming to diminish the perceived barriers to including and promoting 3Rs work.

PE048

G3RP: Good 3R Practice – Quality Management as a Basis for Animal Welfare

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Abstract
Quality Management Systems (QMS) provide a robust structural framework to foster a Culture of Care in animal experimentation. Mostly a QMS is only focusing on processes and technical issues of single experiments but does not interfere with the 3Rs. To address this, the Austrian 3R Center has developed a consulting tool designed to bring more quality and animal welfare into research.

Our approach is based on standard quality management methodologies and offers a tailored consultation. The G3RP Consulting Process begins by defining clear goals and conducting a thorough gap analysis to assess the current state. Whether focusing on a single experiment or an entire animal facility, this tool pinpoints areas for enhancement and offers practical, actionable guidance.

The G3RP ensures continuous improvement and alignment with the 3R principles embedding animal welfare into every aspect in the research process.

The Austrian 3R Center's Gap Analysis (G3RP) takes a holistic approach to ensure reproducibility and data robustness which always implements animal welfare, Refinement, Replacement and Reduction.

Already tested in Austrian animal experimental facilities the G3RP Gap Analysis provides an easy tool for the improvement of quality standards in lab animal research.

PE049

Is There a Place for Rodent Importation and Quarantine in our Future?

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Abstract
Rederivation is not always feasible for rare, unique strains and can be cost prohibitive. Navigation of health data and husbandry information is intimidating and overwhelming. This process can be tedious, but sharing common information between institutions and standardization of excluded agents can streamline the process. Testing for excluded pathogens can easily occur prior to shipping and final approval. Reviewing health data is a priority, but review of health histories and husbandry practices are equally as important. This data represents a time point in the past and is not reflective of the current health status of the institution or individual animals. Quarantine of imported animals in a protected location is best practice, as is routine testing and treatment for parasites that is effective against pinworms and fur mites. This includes *Ornithyssus bacotti*, a feral rodent fur mite highly prevalent in older facilities. Colony animal fecal PCR upon arrival and during quarantine for pathogens should occur during quarantine. Environmental monitoring may play an important role, but efficacy may be limited by testing fewer cages over several weeks. versus large number of cages over 3–6 months.

Rederivation is ideal and the future of new mouse strain acquisition, as methods and costs improve. However, the proprietary nature of rare strains dictate that discretionary importation, coupled with effective quarantine management will still be required. Successful management is feasible without jeopardizing health status with prudent practices and well-trained personnel. Be vigilant when placing your future institutions' health status at risk to both old and emerging pathogens.

PE050

The Use of Environmental Health Monitoring in Rodent Quarantine

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Abstract
The use of sentinel animals for monitoring the health status of imported rodents was once the gold standard for rodent quarantine management. Our institution has recently eliminated the use of sentinel animals, including quarantine1. Health monitoring of quarantined rodents imported from other institutions is arguably the most critical area for biosecurity. In reviewing health data from exporting institutions, results represent a point in time in the past and does not guarantee current SPF status, nor do they eliminate the margin for human error2. Thus, rodent quarantine demands the most efficacious health monitoring methods available. We compared the use of sentinel animals, feces/fur swab PCR testing of quarantine animals and environmental monitoring. Conceptually, environmental testing is highly effective for screening large number of cages over 3–6 months3. However, our quarantine period averages 8 weeks for 2–4 cages per shipment, each with 1–2 adult mice. We used REPLACE (TM) for our environmental substrate and the SFSB for each group. IDEXX BioAnalytics, Inc. performed comprehensive testing of Opti-spots, feces/fur swabs and environmental submissions. With an initial sample size of 10 shipments, test results were conclusive. The results for feces/fur swab PCR testing versus REPLACE substrates were identical. In

one case, both effectively detected MNV while sentinel serology tested negative. These results are very encouraging, since rodent quarantine serves as the gatekeeper safeguarding SPF institutional status. We conclude that environmental monitoring is a highly reliable and reproducible method that effectively monitors the health status of small populations of rodents with shorter periods of exposure.

PE051

Stepping in Front of the Curtain – Making Biomedical Research Tangible for Pupils

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Abstract
Transparency in animal testing will become more and more important in the future. But how should we handle this delicate topic? Which should be the first steps to become transparent? Where should we start? The Department for Biomedical Research – the institution for animal research and alternative methods at the Medical University of Graz (Austria) started their own transparency project by actively inviting schoolchildren from an age of 14–17 years for workshops directly at the Medical University. Teachers are supplied with information material such as a Q&A folder, which was specially created for this purpose, in advance to prepare their pupils for the visit. During the workshop, the children are informed about animal testing, alternatives to animal testing and the 3Rs via presentations and are able to take part in a virtual tour through the animal facility. Afterwards they can test their pipetting skills, do some hand on trainings in sewing and blood withdrawal on dummy models and are able to talk to an animal caretaker to get information about a daily life of a lab animal. Children are our future researchers and we think it is important to start being transparent at an early age. The aim of this workshop is not to convince them what is right or wrong but to support them to set up their own point of view when dealing with a controversial topic like animal experiments. It is time for us to step out of the curtain!

PE052

The Evolution of Laboratory Animal Science Policies in Europe and Brazil: A Bibliometric Analysis

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Abstract
In this paper, we provide a holistic view of the historical development of publications on laboratory animal science across different dimensions, including authorship pattern, keywords, and co-authorship network analysis, to evaluate the growth and the focus of this research field. Some of the most frequently used terms are anxiety, stress, and systematic review, which suggest a particular focus on ethics and animal welfare. Among the top authors are Olsson, Anna (11), Sandoe, Peter (8), and Franco, Nuno (7), because they have made significant contributions to the field.

The work by these authors has influenced debates on ethical aspects and welfare concerning laboratory animal research. The co-occurrence of "anxiety" and "stress" confirms their centrality and impact, thus highlighting the importance of these topics in laboratory animal science. Collaboration networks demonstrate the most important partnerships and institutional connections, and normalized author collaboration networks provide the structural dynamics that underpin knowledge production. This study illustrates how laboratory animal science has developed in response to changing research priorities and societal expectations; it provides a valuable perspective on the field's evolution. An understanding of how research is organized offers actionable insights for the development of research groups and collaborations because it allows for the identification of key areas of focus and opportunities for growth. This work has particular relevance for the further development of the principles of 3Rs and for the alignment of research practices with ethical and societal demands, therefore it has significant implications for the future of laboratory animal research.

PE053

Integrating AI in Animal Welfare: A Chatbot for Humane Endpoints in Laboratory Research

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Abstract
Humane endpoints are designed to improve animal experimentation and reduce animal pain. It has been shown that with precise, humane endpoints, the quality of the results can be maintained while minimizing animal suffering. However, humane endpoints are difficult to define and implement because researchers are required to subjectively determine them, thus making the process challenging. To address this, we developed an innovative chatbot utilizing large language models (LLMs) to assist researchers in the real-time assessment and application of humane endpoints.

The chatbot integrates existing databases, humane endpoint guidelines, and experimental parameters, offering tailored recommendations. It can assess clinical and behavioral data, suggest intervention points, and score endpoints based on severity and scientific justification. The system is trained on various animal models, including rodents and zebrafish, and incorporates inputs from veterinary experts and animal welfare specialists.

Initial validation involved retrospective analysis of pre-existing datasets from animal studies, achieving over 90% concordance with expert assessments. Deployment in active research environments demonstrated its ability to reduce decision-making time and improve the consistency of endpoint applications. It also educates and explains why each tip is beneficial.

This chatbot represents a step forward in integrating AI into laboratory animal science, aiming to standardize humane endpoint practices and improve animal welfare outcomes. This project uses

technology and responsibility to improve the humaneness and safety of animal experiments, and it is based on the 3Rs principles. They provide a framework for improving the welfare of animals used in experiments. Therefore, they are essential for this project.

PE054

Mapping the Educational 3Rs Activities by Developing a Database of Training Programs in Europe

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Abstract
Further development of the 3Rs mainly depend on effective and interconnected 3Rs Education. Operational educational strategies, relay on an objective and updated viewpoint. This initiative is a part of COST Action 'Improving the Quality of Biomedical Science with 3Rs Concepts' (IMPROVE), Working Group 4 – Education. The development of comprehensive database of 3Rs-related educational activities, resources and training programs is currently an ongoing project; it will provide a much-needed actual picture of the current status of 3Rs education in European countries. Thus far, the database includes training program in 17 European, and a total number of 275 training programs. Notably, the most of the recorded training programs are stand alone programs, only few of the them were integrated in other teaching programs, mostly academic. Some programs are dedicated to replacement focusing mainly to MD and PhD students, while the others are dedicated to Education & Training in laboratory animal science. The most of LAS E&T programs are customized for early carrier scientists, veterinarians, laboratory technicians etc., the large majority are certificated at national level of by FELASA. The need of certification made the LAS E&T programs more homogenous in curricula, while the replacement oriented ones are more divers. In brief, they are focused on cell culture, "in vitro" toxicity testing, organoids, 3D cell culture, tissue & organ-on-a-chip, "in silico" models, and, almost all, include topics related to ethics and legislation. Overall, those preliminary data suggest the need of better integration the 3Rs education initiatives.

PE055

Multicolor Flow Cytometric Approach to the Functional Analysis of Cryopreserved Mouse Sperm

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Abstract
As the generation of genetically engineered mice has expanded massively, maintaining these lines has become increasingly important. While gamete cryopreservation is well established, testing sperm quality post-thaw in line with the 3R principles — without using *in vitro* fertilization tests and thus oocytes — remains a significant hurdle. Multicolor flow cytometry enables the multiparametric assessment of the functional status of spermatozoa. This study aimed to establish and optimize a five-color panel for the flow cytometric analysis of frozen-thawed mouse sperm.

Spermatozoa from C57BL/6J males were cryopreserved with the Jax Sperm Cryo Kit. After thawing (37°C), sperm was diluted with modified Krebs-Ringer's bicarbonate medium and stained with calcein violet (1.21 μM), Fluo4-AM (2 μM), phycoerythrin-conjugated peanut agglutinin (PE-PNA; 1 mg/mL), propidium iodide (2.99 mM) and MitoProbe^{TM} DiIC1(5) (0.015 μM) to quantify sperm subpopulations with high esterase activity, low intracellular Ca^{2+} levels, intact acrosome, intact plasma membrane, and high mitochondrial potential, respectively. The fluorescent signal of the respective dyes was captured by a 450/45 (violet laser), 525/40, 585/42 and 690/50 (blue laser) and the 660/20 (red laser) bandpass filter of a CytoFlex flow cytometer. Sperm populations exhibiting up to five functional traits were identified. Staining patterns were consistent across males.

By minimizing the need for live female mice, this method aligns with the 3R principles and holds potential as a reliable, objective tool for sperm quality control in genetic preservation programs. Further validation with a larger sample set and comparison to IVF outcomes are required to strengthen its applicability for routine quality assessments.

PE056

'Green' and Sustainable Vivaria Solutions: Tips and Tricks

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Abstract
Vivaria are some of the most expensive infrastructure investments to build, maintain, and repair. Additionally, vivaria operations are labor intensive activities for a good reason; therefore, there are potential health and safety concerns. This presentation will outline practical and sustainable vivarium solutions to provide 'green', cost effective, and safe operations (for humans and animals) to optimize animal care and provide a pleasant working environment.

PE057

The Benefit of Peripheral Blood Mononuclear Cell Mice Model for Advanced-Stage Lung Cancer

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Abstract
Humanized PBMC mouse models play a crucial role in researching treatments for various types of cancer. However, these models frequently develop xenograft-versus-host disease (xeno-GVHD), which can hinder their effectiveness and result in inconclusive findings. To better understand the potential benefits of these models, particularly in non-small cell lung cancer (NSCLC), we investigated xeno-GVHD responses using PBMCs from advanced-stage NSCLC patients compared to those from healthy donors in a humanized PBMC model. PBMCs from NSCLC patients and healthy donors were injected into immunocompetent NSG-SGM3 mice and monitored for eight weeks. We assessed the progression of xeno-GVHD through clinical examinations of the mice and flow cytometry to measure human T-cell levels in various tissues. Mice injected with PBMCs from healthy donors exhibited earlier signs of xeno-GVHD, while those receiving PBMCs from NSCLC patients showed minimal symptoms, with only one model demonstrating delayed responses. Symptoms observed included weight loss, anemia, low platelet counts, changes in fur, and altered behavior. Flow cytometry results indicated a predominance of CD8+ effector memory T cells, with overall T-cell levels being lower in the NSCLC models than those from healthy donors. Our study highlights significant differences in the progression of xeno-GVHD in NSCLC patients, likely influenced by their treatment histories. We identified several clinical and cellular markers associated with early xeno-GVHD responses in mice. Furthermore, our findings enhance the understanding of humanized PBMC models in NSCLC, which could inform future treatment studies, improve patient selection, and refine the use of these models.

PE058

A Comparison of Three Viral Infections with Radiomics; What Can't We See?

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Abstract
SARS-CoV-2, pandemic H1N1 (pH1N1) and avian influenza virus (H5N1) can all cause disease in humans, all show differences in cytokine profile and lung CT. The aim of this study is to quantify and investigate those differences using radiomic texture features.

Retrospective analyses, on previous ethically approved studies, were executed on data [1–3] from cynomolgus macaques (*Macaca fascicularis*) inoculated with either influenza A/Mexico/InDRE4487/2009 (pH1N1) virus (n = 4) or influenza A/Vietnam/1203/04 (H5N1) virus (n = 4) or rhesus macaques (*Macaca mulatta*) with BetaCoV/German/BavPat1/2020 (Wuhan strain D614G) (n = 6). Blood sampling and CTs were performed before infection and on day 2, 7 and 11/14 post infection. The whole lungs were, based on the CT, selected as region of interest and loaded in LIFEx v7.5.0 [4] to perform texture analysis.

The infections differed in several features. The steep increase of the Intensity Variance within the lungs of H5N1 proved to be a hallmark of the fast development of lesions and severity of the infection compared to the other two infections. The Intensity Kurtosis represents a measure for the nature and density of the lesions with consolidations versus ground glass opacities. Combining these two, together with the cytokine profiles of IL-6 and PLT, allowed us to differentiate between all three infections.

This shows that radiomics can be used as method for an in-depth description of lung CTs. In addition, when comparing features across models and timepoints, differences could be identified which allow discrimination between these models. Evidence is provided for the role of texture analysis in pre-clinical infectious models.

PE059

Cutting-edge Cannabinoid Approaches for Alzheimer's: A Future-Oriented Research Perspective

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Abstract
The increasing global prevalence of Alzheimer's disease (AD) highlights the urgent need for novel therapeutic approaches in both treatment and research. Conventional therapies, such as acetylcholinesterase inhibitors, offer only limited symptomatic relief, emphasizing the necessity for more comprehensive and multifaceted solutions. This study investigates the potential of combining cannabinoids with established reference compounds for AD, focusing on the entourage effect, whereby cannabinoids exert synergistic interactions to enhance therapeutic efficacy. This multi-target strategy aims to address the cognitive, metabolic, and motor deficits characteristic of AD.

In addition to exploring innovative therapeutic avenues, this research also emphasizes a forward-thinking approach to AD studies by prioritizing ethical considerations in animal experimentation. By utilizing transgenic AD mouse models, we aim to deepen our understanding of the disease's underlying mechanisms and therapeutic potential, while minimizing invasive procedures and prioritizing animal welfare. This approach underscores the translatability of cannabinoid-based therapies, bridging preclinical research with clinical applicability.

Ultimately, this study is driven by the vision of a better future, where groundbreaking therapies not only improve clinical outcomes for AD patients but also foster more ethical and sustainable research practices. By integrating cannabinoid-based treatments into AD care, we seek to promote a more ethical, effective, and sustainable approach to neurodegenerative disease research, ensuring that scientific advancement is coupled with the reduction of animal suffering.
Funding: This research was funded by a grant of the Ministry of Research, Innovation and Digitization, CNCS - UEFISCDI, project number PN-IV-P2-2.1-TE-2023-0879, within PNCDI IV.

PE060

Systematic Online Living Evidence Summary (SOLES) For the Pre-clinical Testing of Targeted Cancer Therapies

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Abstract
Cancer is a leading cause of death worldwide and new therapies are needed, necessitating testing in animal models. Although a wide variety of tumor models have been described in the literature, their limitations, as well as unsuccessful translation attempts are often not reported. Yet, choosing the right animal model for the drug to be tested and successfully translated into the clinic is critical.

We systematically reviewed studies testing targeted therapies with the goal to find animal models which have been successfully tested in cancer research and to identify variables that contribute to successful translation and markers of high external validity.

Given the substantial number of studies potentially relevant to this topic, we are developing and validating an automated approach to categorize and prioritize studies prior to in-depth review of disease domains with potential for greatest impact. We will establish and validate the approach with a manageable number of publications, to demonstrate the feasibility of extension to other tumor entities.

Results will be implemented in a systematic online living evidence summary (SOLES), an interrogatable database accessed through a user-friendly online dashboard. The dashboard will be used to filter studies based on study design features of interest, supporting the conduct of specific systematic reviews and highlight gaps in the literature for future research. The Cancer-SOLES platform will provide novel opportunities to identify drug and model combinations that show concordance with human outcomes, optimize the model selection process for preclinical cancer studies and to reduce the number of animals used in cancer research.

PE061

Responsible and Efficient Animal Research Through Digital Resource and Access Management

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Abstract
"Enhancing precision and transparency" is the guiding principle in the digital transformation of our animal research facility, aiming to create a research environment that centralises both animal welfare and efficient resource management. Through a fully integrated digital infrastructure, we will establish an ecosystem where microchipping, RFID readers, and automated access controls converge to support resource optimisation and traceability at every level of animal care.

Microchipping each animal, combined with RFID tracking, enables precise identification and real-time monitoring, reducing handling time and ensuring minimal stress for the animals. Each team member gains access to the system via individual tokens on a touchscreen console, required before beginning any procedure, thereby ensuring comprehensive security and an auditable interaction log. This digital infrastructure further extends to home cages, where automated inventory systems maintain a real-time record of animal counts, health certifications, and acquisition details, promoting a consistent and traceable workflow.

This setup not only alleviates administrative burdens and reduces potential errors but also enhances accountability and compliance with regulatory standards. By combining advanced digital tools with responsible research practices, we foster an environment where each aspect, from resource allocation to animal welfare, is seamlessly managed and traceable. Our approach aims to demonstrate how digitalisation can elevate animal research practices by optimising resources and setting new standards for transparency, accountability, and ethical responsibility in the field.

PE062

Establishment of a Severely Immunodeficient NOG Mouse Strain Exhibiting a Short-limbed Phenotype

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Abstract
Short-limbed mice were found in a NOG brother-sister mating colony. The morphological abnormalities were revealed by pathological and image analysis, and mating tests and phenotypic analysis suggested that the causative gene was a recessive genetic trait. Based on this information, a comprehensive database search was conducted to narrow down the candidate causative gene to Gdf5, and a gene mutation in the Gdf5 gene was found due to sequence analysis of the Gdf5 gene. As a result, the recessive mutant trait was eliminated from the NOG brother-sister mating colony. In inbred brother-sister mating of mice, attention must always be paid to the expression of recessive traits, the detailed understanding of the morphological abnormalities by pathological and image analysis, and the method of narrowing down the causative gene by comprehensive data search based on that information, are highly effective for the maintenance and production of inbred mice.

PE063

Automated Home-cage Monitoring: Bridging Refinement and Sustainability in Animal Research

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Abstract
Refinement in mouse experimental research is an ongoing task aimed at improving animal welfare. It involves applying measures before and during experiments to minimise discomfort and ensure humane endpoints. This principle has long been a cornerstone of responsible research.Meanwhile, sustainability—focused on reducing ecological footprints and minimising environmental harm—has recently emerged as a critical objective. Achieving zero-carbon laboratories is a key goal in this context. While refinement and sustainability may initially seem unrelated, automated home-cage monitoring systems offer an effective way to integrate both principles.

Automated systems, such as the Digitally Ventilated Cages (DVC; Tecniplast), enhance refinement by continuously tracking mice activity, particularly at night. This enables precise identification of discomfort and early determination of humane endpoints, improving animal welfare.

Sustainability benefits stem from optimising cage management. By accurately determining when cage cleaning and bedding changes are needed, these systems reduce bedding material use and decrease the number of washing and autoclaving cycles. Consequently, resource consumption, energy use, and carbon emissions are significantly lowered.

Our experience with automated home-cage monitoring demonstrates that it is possible to refine animal testing while simultaneously contributing to sustainability. This dual impact is a crucial advancement in modern research practices.

In summary, automated monitoring systems serve as a vital bridge, harmonising refinement and sustainability—formerly perceived as separate goals—into a unified approach that benefits both animal welfare and environmental responsibility.

PE064

Exploring Recombinant Bacteriocins in a Murine Model of Klebsiella pneumoniae Infection

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Abstract
Antimicrobial resistance represents a significant global challenge, with *Klebsiella pneumoniae* exhibiting alarming resistance rates of up to 70% and infection-related mortality rates ranging from 40% to 70%. Traditionally managed using β-lactams and other antibiotics effective against Enterobacteriaceae, antibiotic-resistant *K. pneumoniae* has become increasingly prevalent across diverse populations, underscoring the urgent need for novel antimicrobial therapies. Recombinant bacteriocins, such as klebicin KvarM and chimericin ColA-Kvarla, are emerging as potential alternatives for combating drug-resistant bacterial infections.

The primary aim of this study was to develop a *K. pneumoniae* infection model in mice (C57BL/6J, n = 5 per group) and evaluate the efficacy of antimicrobial agents in treating intestinal infections. Using fecal samples collected at multiple time points during the experiments, bacterial colonization and treatment efficacy were assessed through DNA extraction, RT-qPCR analysis, amplicon preparation, and sequencing of 16S rRNA gene V1-V2 regions.

The study demonstrated the successful establishment of an in vivo infection model using the commercial *K. pneumoniae* strain ATCC 43816™. Both KvarM and ColA-Kvarla were effective in significantly reducing bacterial infection, performing comparably to ciprofloxacin. Importantly, KvarM and ColA-Kvarla specifically targeted *K. pneumoniae* without disrupting the natural gut microbiota, a notable advantage over antibiotics, which broadly impacted microbial diversity and composition.

In conclusion, klebicin KvarM and ColA-Kvarla show promise as targeted antimicrobial agents for treating antibiotic-resistant *K. pneumoniae* infections. These findings are preliminary and warrant further validation in future research.

PE065

Home Cage Stereotypic Behaviors Affect Behavioral Phenotypes and are Modulated by Environmental Enrichment

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Abstract
The display of stereotypic behaviors (SB) is ubiquitously observed in standard housing of laboratory mice in shoe box sized cages with limited options to satisfy the animals' drive for exploration, physical and social activity.

There is ample evidence for a positive effect of environmental enrichment (EE) on home cage SB. However, we have hitherto lacked the technical options to continuously monitor larger cohorts of animals over prolonged periods of time in their home cage to determine SB and the effect of EE in an unbiased manner. Current methods mostly rely on the manual evaluation of video footage of a limited number of animals during specific temporal epochs.

We here used the Tecniplast Digitally Ventilated Cage-System® to develop an algorithm for the automated and continuous detection of circling behavior, a frequent home cage SB. We monitored over 200 mice of both sexes under varying housing conditions in

different experimental cohorts and determined home cage SB and paralleling performance in standard behavioral paradigms.

Overall, we found low levels of SB that would have likely escaped conventional evaluation methods, with females performing significantly more stereotypic circling than males. Levels of SB were associated with alterations of phenotypes in standard behavioral tests. Social and environmental factors of the home cage modulated SB and the results of behavioral testing.

We conclude that the display of SB can be modulated by the environment of the home cage and that even low levels of SB can bias study outcomes and increase experimental variability.

PE066

The Development of a Three-stage Training Program on Performing Non-Clinical Studies in Lab Animals

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Abstract
Objective(s): A training program based on the FELASA-LAS course was designed to perform nonclinical studies of high-quality data using lab animals.
Introduction: Non-clinical studies are crucial in drug development, assessing safety & efficacy before human exposure. These studies must comply with Good Laboratory Practice (GLP) principles and regulatory guidelines of OECD, ICH, FDA and EMA. Data quality, reliability, consistency, integrity and traceability are prerequisites. Achieving this requires: i) well-maintained, fully equipped facilities with monitored environmental conditions ii) a study plan approved by local authorities iii) appropriate species selection with ethical compliance iv) proper documentation per Standard Operating Procedures (SOPs) v) accurate reporting of results, and vi) well-trained, qualified personnel. Our team developed a training program to ensure personnel involved are qualified to generate reliable and reproducible data.
Materials & Methods: FELASA-certified personnel underwent a three-stage training course. In zero-stage, trainees were instructed in basic techniques for handling, restrain, drug administration and fluids collection in CD-1 and C57BL/6 mice and Wistar rats, following the FELASA-LAS curriculum. Stage-I involved repeating specific techniques (e.g., oral gavage, IV injection, sparse blood sampling) in a different cohort to standardize procedures. Normal saline was administered in both stages. In stage-II, trainees simulated a pilot pharmacokinetic study, performing single-dose oral and IV saline administration. Study-related activities (e.g. identification, weighting, dosing, blood sampling) were documented and trainee qualification process was implemented throughout the program.
Conclusion(s): The three-stage program provides a stepwise approach to develop highly skilled personnel in animal handling, treatment and blood sampling, ensuring high-quality non-clinical data.

PE067

Patient-derived Xenograft and Organoids Models for Study of Anticancer Drugs Efficacy

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Abstract
Pancreatic cancer is a severe oncological disease with an ever-increasing incidence and a high rate of morbidity and mortality. Therapeutic options are limited and the five-year overall survival rate is 7–20%, depending on the possibility of surgical resection and the earliness of detection. Most patients with this diagnosis die due to the resistance of tumour cells and their microenvironment to the used treatment regimes. The aim of this study was to implement the patient-derived xenograft (PDX) in vivo models of pancreatic carcinomas into the study of anticancer drug efficacy and start to develop patient-derived organoids (PDO) as 3D miniaturized versions of tumors with functions of their in vivo counterparts. We successfully created three PDX models with various subtypes of pancreatic cancer (PDAC, APC and ASPC). These tumours were re-transplanted into subsequent generations of NU/NU mice and then used to compare conventional and experimental chemotherapy regimens. Furthermore, we also started to establish organoids from tumor cells of pancreatic carcinoma patients. Optimization of organoid culture conditions is currently underway. In conclusion, the established PDX or PDO models serve us not only for the search for optimal therapeutic regimens but also for future discovery of signaling pathways behind the drug resistance and aggressiveness of pancreatic carcinoma.
Supported by the Czech Ministry of Education, Youth and Sports, INTER-ACTION LUAUS23164, National Institute of Public Health (institutional support to A.S.), the NICR project LX22NPO5102 financed by European Union – Next Generation EU as part of the Czech Recovery Plan, and and GAUK No. 308223.

PE068

Integrating Fluorescence Bioimaging and LC-MS for Refining Animal-Based Pharmacokinetics Studies

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Abstract
The development of alternative strategies for the reduction and refinement of animal experimentation is a critical component of the 3Rs principles in animal welfare. This study explores the synergy between fluorescence in vivo bioimaging and liquid chromatography-mass spectrometry (LC-MS) as complementary methodologies for analyzing the pharmacokinetics and biodistribution of chemotherapy drugs in biological samples and tissues. Fluorescence in vivo bioimaging provides real-time, non-invasive tracking of drug distribution and accumulation, enabling temporal monitoring of pharmacokinetic profiles in living organisms. However, a key limitation of fluorescence bioimaging is its inability to differentiate potentially inactive fluorescent metabolites from the original drug, which could lead to ambiguity in interpreting results. This limitation is effectively addressed by LC-MS, which offers high sensitivity and precision in identifying and quantifying both parent compounds and their metabolites across various biological matrices. By integrating these techniques, we demonstrate how both approaches can be used to reduce the need for repeated animal sampling, minimizing animal usage and stress, and refining experimental protocols. This combined approach allows for continuous, non-invasive monitoring of drug behavior over time, significantly enhancing the understanding of drug pharmacokinetics. Additionally, it facilitates the assessment of drug localization in specific tissues without the need for extensive biopsies, further reducing animal use. Our findings support the potential of fluorescence bioimaging and LC-MS as powerful, complementary tools in pharmacokinetics studies, providing more accurate and detailed data while aligning with the principles of the 3Rs in the context of chemotherapy drug evaluation and broader pharmaceutical research.

PE069

Visitor Survey at the Open Day

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Abstract
On our poster -"With brain and heart - insights into our modern animal facility" - Visitor survey at the open day- we present how the work with laboratory animals is perceived by the public.

We celebrated the open day at the DZNE Bonn on 16.06.2024. At our presentation stand, we collected information on which areas the visitors come from, what points of contact they have with animal experiments and how this may influence their view of animal experiments. We also wanted to find out how the presentation of our animal facility, with photos from the husbandry, at the stand (film, poster and exhibition of transfer station, enrichment and cage rack as a hands-on activity) was received by the visitors.

The evaluation of the survey is shown on the poster.

The aim of our the survey was to find out how we can improve external communication in the future.

How can public relations work help to create more acceptance and awareness among the population?

PE070

Evaluating Animal Models to Unravel the Complexities of Post-acute Sequelae of COVID-19

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Abstract
Although the COVID-19 pandemic, caused by a SARS-CoV-2 infection, is no longer classified as global health emergency, a significant number of patients continue to suffer from long-term effects, known as post-acute sequelae of COVID-19 (PASC). To understand the complex pathophysiology of PASC in a controlled setting, animal models that replicate the disease's post-acute phase are essential.

Our review explores the status of animal models studied beyond the acute phase of SARS-CoV-2 and discusses the added value of different models. After screening 1977 abstracts, 92 studies were summarized in an overview table, with 9 studies monitoring animals for at least 3 months post-infection. Mice, Syrian golden hamsters and non-human primates were identified as the most used models.

Innovative technologies continue to emerge, also artificial models were included using patient fecal transplants, the passive transfer of IgG antibodies or the infusion of the SARS-CoV-2 Spike protein into the brain to mimic and better understand PASC.

Several possibilities are hypothesized as driving force of different symptoms of PASC, such as prolonged inflammation, but no single model replicates all of them. However, studies performed in animal models indicate that multiple hypotheses can be applied to study PASC. It is likely that there is not just one hypothesis correct but that several hypotheses are associated with each other. Animal models will help us to link these hypotheses together and hopefully unraveling the complexities of PASC. Nevertheless, to achieve this, further research is necessary to fully bridge the gap between animal studies and clinical insights.

PE071

Integrating Education to Advance FCS-free Cell Culturing; a Collaborative Approach in Laboratory Animal-Free Research

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Abstract
In line with the 3Rs principle, the 3Rs Centre Utrecht and the Utrecht University of Applied Sciences have developed innovative educational research projects. These challenge-based initiatives aim to replace animal-derived components in cell culture media, focusing on alternatives to fetal calf serum (FCS), while creating an authentic research environment for students. This integration of education enhances scientific training and increases the project's impact by mentoring a new generation of researchers dedicated to animal-free science. FCS, derived from fetal calves, raises ethical concerns and can compromise experimental quality and reproducibility due to its undefined composition.

In 2024, third-year bachelor students enrolled in the Innovative Laboratory Animal-Free Methods course and tested serum-free alternatives in routine in vitro cellular models for muscle development (C2C12), cancer (DAOY), gene/protein expression studies (HEK293), and the immune system (THP-1). Tested alternatives included commercial media (Proliferum M and Panexin Basic) and a defined non-commercial mediumA, B.

The results were mixed. Proliferum M supported C2C12 proliferation but still required horse serum for differentiation. DAOY and THP-1 cells exhibited poor growth with Panexin Basic, while the defined medium allowed a 50% reduction in FCS for HEK293T and THP-1 cells without compromising morphology. Further studies will evaluate functional performance and freezing/thawing effects under serum-free conditions.

Supported by the FCS-free databaseC and collaborations at Utrecht Science Park, this project demonstrates the potential to reduce serum dependency in routine cell culture. It underscores the importance of integrating education, research, and industry collaboration to further contribute to global 3Rs efforts and improving experimental reproducibility.

PE072

Influencing the Discussion on Moving Away from Animal Testing; the Role of Policy Makers

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Abstract
In line with European policy ambitions, government programs on reducing animal testing have been running for several years in the Netherlands and Flanders (Belgium) and policy makers are working closely with the National and regional Committees. Despite the cooperation of various stakeholders from within the animal research community, the transitioning away from animal experimentation is often met with skepticism. We believe that policy makers can make a difference. Transitions commonly progress through distinct phases, requiring policymakers to align their strategies to respond to shifting dynamics Depending on the phase of the transition, they have to take on a different position and different tasks. An international policy maker network could assist policy makers in this and would bring the European ambition closer.

PE073

In Vitro and In Vivo Efficacy of New Taxane-based Chemotherapeutics in Resistant Ovarian Carcinoma

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Abstract
Taxanes are widely used in the treatment of ovarian carcinomas. One of the main problems with conventional taxanes is the risk of development of multidrug resistance. New-generation synthetic experimental taxoids (Stony Brook Taxanes; SB-Ts) have shown promising activity against various resistant tumor models. The aim of our study was to compare the in vitro potency, intracellular

content, and in vivo antitumor effect of clinically used paclitaxel (PTX) and SB-Ts from the previously tested second-generation (SB-T-1214, SB-T-1216) and the newly developed third-generation (SB-T-121402, SB-T-121605, and SB-T-121606) SB-Ts in PTX-resistant ovarian carcinoma cells NCI/ADR-RES. The potency of the new SB-Ts was up to 50-times higher than that of PTX in NCI/ADR-RES cells in vitro. SB-T-121605 and SB-T-121606 induced cell cycle arrest in the G2/M phase much more effectively and their intracellular content was 10–15-times higher than that of PTX. Incorporation of SB-T-121605 and SB-T-121606 into therapeutic regimens of PTX was effective in suppressing tumor growth in vivo in NCI/ADR-RES mice xenografts at small doses ($\leq$3 mg/kg), wherein their adverse effects were not observed. In conclusion, new SB-Ts, SB-T-121605 and SB-T-121606, are promising candidates for the next phase of preclinical evaluation against resistant ovarian carcinomas in combination with conventional taxanes. This work was supported by the Czech Ministry of Education, Youth and Sports, INTER-ACTION LUAUS23164, Grant Agency of the Czech Republic no. 21-14082S, NICR project LX22NPO5102 financed by European Union – Next Generation EU as part of the Czech Recovery Plan, and the National Institutes of Health (NIH), U.S.A. grant R01 CA103314.

PE074

GLP Toxicity Assessment of Novel Intranasal Adenovirus-based COVID-19 Vaccination, COV2, in Rat

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Abstract
Efforts to develop intranasal vaccinations for COVID-19 disease may offer a better prevention by accomplishing a local IgA antibody immune response against the viral particle upon entry in addition to systemic IgG response. This GLP study investigated the toxicity and immunogenicity of a novel adenovirus-based COVID-19 vaccine (COV2) after three intranasal administrations given at two-week intervals to the Sprague-Dawley rats. The study contained main study group euthanised two days after and a recovery group euthanised two weeks after the last dosing event. The calculated human equivalent dose (HED) for was approximately 26 times the actual human dose in females and 13 times in males. The COV2 vaccine elicited IgG immunogenic response in the rat. COV2 was associated with slight reduction in body weight gain in female animals towards the end of the study when comparing to saline control animals. COV2 administration was associated with transient histological changes in nasal cavities. Pulmonary histological findings were only partially recovered and were likely associated with unintended deposition of the test item in the pulmonary parenchyma. In conclusion, three intranasal administrations of COV2 in the rat were well-tolerated and did not cause mortality or toxicity related clinical signs during the in-life period of the study. The candidate molecule COV2 has proven to be safe and well-tolerated and will be used in human clinical trials.

PE075

Improving Translatability of Preclinical Stroke Models Through Systematic Review: Focus on Biological Sex

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Abstract
Ischemic stroke is a leading cause of global disability, yet its precise pathogenic mechanisms remain elusive, limiting the effectiveness of current treatments. Preclinical stroke models often fail to consider critical patient characteristics such as age, health status, and sex, potentially leading to translational failure. Preclinical systematic reviews offer a robust evidence synthesis method to identify knowledge gaps and inform future animal experiments. Understanding sex differences in stroke is essential for improving the translatability of preclinical research to human patients and ultimately reduces the total number of animals required thereby supporting the 3Rs principle. Our systematic review focuses on sex differences in stroke outcomes and treatment efficacy, providing insights into distinct pathophysiological pathways and therapeutic responses. Such evidence is pivotal for developing effective, personalized interventions for all genders. Systematically searching three bibliographic databases and using machine learning we identified 4896 unique records. These were screened by two independent reviewers, resulting in the inclusion of 154 studies using both sexes to model focal cerebral ischemia. Preliminary meta-analysis revealed that females have 34% (95% CI: 43.5–24) smaller infarcts than males, though this advantage was absent in aged and ovariectomized animals. No sex differences were found in post-stroke survival rates. Further analysis will explore mechanisms behind these sex differences and assess the impact of effect modifiers, such as stroke induction techniques. By addressing these gaps, our work aims to refine preclinical research practices, improve model translatability, and advance the development of more inclusive and effective therapies for ischemic stroke.

PE076

Development and Characterization of a Large Colony Cage for Rat Toxicity Studies

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Abstract
Rodent toxicity studies are mandatory (ICH, OECD) to characterize the safety risk of chemicals or pharmaceuticals and are conducted mostly with rats housed in small groups. To refine housing, we

developed novel colony housing systems that provide rats a more species-specific environment with a basis for their social behavior.

In a standard 4-week oral toxicity study with male Wistar rats (n = 15/group), we compared head-to-head the study feasibility in Typ-IV cages (6 × 2–3 rats), a rat colony cage (RCC) consisting of 4 levels vertically connected by jump holes or a staircase (15 rats) and a tox rat colony cage (TRCC) that comprises 4 type IV cages flexible connected horizontally (15 rats). Rats were treated with the antirheumatic/chemotherapy compound methotrexate (0.2 mg/kg/d) or vehicle.

We demonstrated that both colony cage types were very convenient for rats, animal care takers and for the collection of all endpoints. RCC and TRCC animals gained more body weight allowing stronger discrimination of mild treatment effects. No cross contamination with the test item was detected. Interestingly, male reproduction organs showed higher (double) weight variability in the RCC compared to type IV or TRCCs. Finally, methotrexate induced suppression of immunoglobulin A serum levels, that are known to be also stress modulated, recovered faster in colony cages.

In conclusion, standard toxicity studies can be done in large colony housing systems allowing full data collection and a more species-specific environment. The TRCC design is most flexible, does not increase organ weight variability and is very suitable for refinement in rodent toxicology.

PE077

Impact of Bacteria on Diet-induced Obesity Development and Type 2 Diabetes Treatment in Mice

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Abstract
The health status of laboratory animals and the impact of microorganisms on experiments are of great importance to animal welfare and the generation of scientifically valid data in animal experiments. Different microorganisms are common in laboratory animal husbandries and usually do not cause symptoms in immunocompetent animals. However, their influence on test results is often unknown. For disease models, the possible influences of the animals' microorganisms should therefore be taken into account.

A frequently used model is the diet-induced (DIO) obesity model with male C57BL/6J mice. It is used to study metabolic diseases including type 2 diabetes. The steady increase in obesity and diabetes patients worldwide requires research into prevention and therapy.

In a study with experimentally infected DIO mice, we investigated whether pathogens that frequently occur in laboratory animal husbandry and are also relevant for diabetes patients affect the development of pre-diabetes and treatment with incretin analogues.

Male C57BL/6J mice were experimentally infected with *Helicobacter hepaticus, Rodentibacter pneumotropicus* and *Staphylococcus aureus* and monitored over a period of 26 weeks of high-fat diet feeding. In addition, mice were then treated daily with GLP-1 receptor agonist liraglutide or the GLP-1/GIP co-agonist MAR709. Both feeding and treatment were compared to a group of uninfected specific and opportunistic pathogen free (SOPF) mice. Our data show that the DIO model under bacterial colonization with these agents might be accepted for the investigation of metabolic disorders such as obesity and diabetes.

The study and selected results will be presented, and conclusions drawn for diabetes models.

PE078

Dietary Ingredients Impact Background Fluorescence Signals during In Vivo Imaging in Mice

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Abstract
In vivo fluorescence imaging is a powerful non-invasive tool to assess whole-body distribution of fluorescent probes in diverse research models. However, a significant limitation is the background fluorescent signal of certain dietary ingredients like chlorophyll-containing alfalfa commonly found in grain-based chow diets. Purified diets made with refined ingredients eliminate this background enabling a more accurate imaging signal. This study evaluated the intensity of gastrointestinal fluorescent signals from dietary ingredients (alfalfa/dyes) and assessed age-related differences in the clearance of these signals in mice consuming alfalfa-containing chows and purified diets, using the IVIS® Spectrum2 imager. Male NU/J and C57BL/6J mice were maintained either on chows (with/without alfalfa) or purified diets to evaluate diet-derived fluorescent signals, while age-related differences in clearance of the fluorescent signal were assessed in 8 or 52 week old male UM-HET3 mice. Alfalfa-containing chows produced substantial fluorescent signals at 640/675 nm, high enough to interfere with typical probes in both NU/J and C57BL/6J mice. In contrast, alfalfa-free diets, including purified diets, with or without dyes, showed minimal fluorescent signals, making them more suitable for imaging applications. While imaging the rate of clearance of alfalfa-containing chows in UM-HET3 mice, age-related differences were observed, with younger mice clearing 90% of alfalfa-derived fluorescent signal within 4–5 days, whereas older mice needed 10–11 days. These findings underscore the importance of proper diet selection in imaging studies, particularly the use of purified diets to reduce background signal, thereby improving the accuracy and quantification of targeted fluorescent probes in vivo.

PE079

Non-invasive Imaging as a Tool for 3R Principles: Assessment of Chemotherapy-Induced Cardiovascular Toxicity

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Abstract

Cardiac ultrasound (echocardiography) is a noninvasive imaging technique that provides real-time information to assess heart structure and function in clinical practice and preclinical research. This study aimed to evaluate the feasibility of in-vivo longitudinal assessment of chemotherapy-induced cardiovascular toxicity in mice.

The alkylating agent cyclophosphamide (Cy) is a cornerstone in cancer treatment. However, the treatment is associated with several adverse effects including cardiovascular complications. We established a mouse model to evaluate Cy-induced cardiovascular toxicity, to test new prophylactic strategies. Mice received Cy at doses of 300–600 mg/kg. Cardiac function was monitored longitudinally, individually, over eight weeks using echocardiography to minimize the inter-individual variability and reduce the animal number. 2D B-mode and M-mode imaging of the left ventricle long-axis view quantified key parameters such as ejection fraction (EF), fractional shortening (FS), cardiac output (CO), and stroke volume (SV). Additionally, speckle-tracking strain analysis was employed for early-stage detection of Cy-induced cardiac toxicity, enabling diagnosis before significant changes in ventricular volumes or ejection fraction are observed. Throughout the experiment, the principle of refinement was upheld by real-time monitoring of multiple physiological parameters, including heart rate, body temperature, respiratory rate, and electrocardiography (ECG), which are significantly influenced by anesthetics. To ensure animal welfare, heating pads, and lamps were used to maintain physiological body temperature.

In conclusion, non-invasive imaging techniques provide significant advantages by enhancing animal welfare and enabling longitudinal analyses. Efficient experimental designs support generating meaningful outcomes using reduced animal numbers and refining the animal conditions that align with the 3R principles.

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