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3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION SESSION

INFRAFRONTIER.GR: ADVANCING THE 3RS IN DISEASE MODELLING

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Recent innovations in genomics, non-invasive phenotyping technologies, advanced *in vitro* and microphysiological systems, as well as regulatory and legislative shifts have accelerated the adoption of the 3Rs (Replacement, Reduction, Refinement) across biomedical research. INFRAFRONTIER.GR, the Greek Research Infrastructure for archiving and phenotyping of model mammalian genomes and the Hellenic node of INFRAFRONTIER ERIC, has played a central role in translating these advances into practical solutions that improve animal welfare while strengthening scientific outcomes.

Through its network, INFRAFRONTIER.GR supports the development, harmonization, and deployment of next-generation disease models and methodologies. Key achievements include optimized cryopreservation strategies that enable efficient archiving and global distribution of mouse lines, substantially limiting the need for breeding and transport of live animals. Standardized sperm and embryo preservation protocols, combined with refined quality control approaches, allow repositories such as the European Mouse Mutant Archive (EMMA) to maximize resource sharing while minimizing animal use. In parallel, INFRAFRONTIER.GR has advanced systemic and disease-oriented (e.g. chronic inflammation, degenerative diseases, etc.) phenotyping services by refining experimental designs, introducing non-invasive and non-terminal tools, improving housing and handling practices and providing standardized, role-specific training pathways. These measures enhance data robustness while reducing severity, supporting more ethical and reproducible *in vivo* studies. Importantly, INFRAFRONTIER.GR is expanding beyond animal-based models by integrating complex *in vitro* systems into its service portfolio. 3D cell cultures, such as organoids, are being leveraged to develop biologically relevant alternatives to mouse models, particularly in gastrointestinal, respiratory and cancer research. Ongoing projects aim to establish reliable *in vitro* platforms for diseases, reducing and complementing animal use in these areas.

Together, these activities demonstrate how coordinated Greek research infrastructures can drive innovation, promote collaboration, and embed the 3Rs at the core of disease modelling.

EVALUATION OF REPLACEMENT METHODS IN NON-TECHNICAL PROJECT SUMMARIES (NTS)

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Article 37 of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes requires that Member States shall ensure that an application for project authorisation is submitted by the user or the person responsible for the project. The application shall include at least the following: (a) the project proposal; (b) a non-technical project summary. Both documents shall provide a demonstration of compliance with the requirements of replacement, reduction, and refinement. The Directive also requires Member States of the European Union (EU) to publish non-technical project summaries (NTS) of all authorized projects involving animals (Article 43).

The study aims to evaluate the alternative methods described by the applicants at the institutional level (University of Debrecen) project proposal and non-technical summaries (NTS), as well as in the international level in animal use reporting EU system available NTS database.

During the study were examined that the person responsible for the project in the replacement part of NTS “explains what partial and/or full replacements were considered – or used – prior to deciding to use animals, and these may include in silico, in vitro, or ex vivo approaches, and explains why they were not (yet) suitable” (1).

References:

1. Taylor K, Weber T, Alvarez LR. Have the non-technical summaries of animal experiments in Europe improved? An update. *ALTEX* (2024). 41(3):382-394. doi: 10.14573/altex.2310181.



ULTRASOUND-GUIDED REFINEMENT OF BOVINE RESPIRATORY DISEASE COMPLEX STUDIES

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Introduction: Bovine Respiratory Disease Complex (BRDC) remains one of the most significant health and welfare challenges in cattle, resulting from complex interactions between viral and bacterial pathogens, host immunity, and environmental stressors. Experimental BRDC challenge models are essential for evaluating vaccine efficacy and advancing understanding of disease pathogenesis. However, variability in baseline pulmonary health can compromise animal welfare and scientific outcomes.

Aims: This work aimed to refine an existing *in vivo* BRDC challenge model by integrating non-invasive diagnostic imaging to improve baseline uniformity, enhance animal welfare, and support evidence-based decision-making, while maintaining scientific quality and reproducibility.

Materials and Methods: Transthoracic ultrasonography was systematically incorporated into animal inclusion and monitoring procedures. Calves underwent comprehensive lung ultrasound screening to identify pre-existing pulmonary lesions or consolidations associated with increased risk of adverse outcomes following challenge. Animals with significant baseline pathology were excluded. Following challenge, ultrasound examinations were repeated at defined time points to assess lung pathology and guide decisions regarding animal retention, intervention, or removal from the study. In parallel, ongoing research is investigating the development of non-invasive microRNA (miRNA)-based diagnostic tests for earlier detection of BRDC.

Results: Ultrasound-guided refinement resulted in more uniform and healthy study cohorts and improved consistency in disease expression across treatment groups. The proactive use of transthoracic ultrasonography enabled early identification of animals at risk of severe disease and has virtually eliminated post-challenge mortalities. In addition, ultrasound provided a rapid, non-invasive method for monitoring disease progression and supporting objective clinical assessment.

Conclusion: Integrating transthoracic ultrasonography represents a meaningful refinement of an existing *in vivo* BRDC challenge model. The use of non-invasive diagnostic imaging reduces suffering and avoids unnecessary mortality by enabling evidence-based decision-making, while improving baseline uniformity, scientific quality, and reproducibility. Complementary miRNA biomarker research offers future opportunities to further refine respiratory disease models and reduce disease burden.



STOP THE COLD BEFORE THE FALL: HEATED ANESTHESIA INDUCTION IN MICE

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Introduction: Peri-anesthetic hypothermia is a common and critical complication of rodent anesthesia, negatively affecting animal welfare, physiologic stability, and experimental reproducibility. Although active warming is routinely applied during anesthetic maintenance, the induction phase (when thermoregulatory defenses collapse and heat loss is most rapid) often remains unprotected.

Aims: To evaluate a cost-effective Arduino-based heated anesthesia induction as a refinement strategy for early hypothermia in mice and to assess its effects on thermal stability and anesthetic physiology.

Materials and Methods: A precisely temperature-controlled, low-cost heated induction chamber was developed to provide active warming at the onset of inhalant anesthesia. Bench testing assessed thermal stability and surface temperature accuracy. In a randomized, paired, repeated-measures design, ten adult C57BL/6 mice (5 females/5 males) served as their own controls and underwent isoflurane induction under two conditions: unheated induction and heated induction (37.5 °C). All animals then underwent a standardized 10-minute anesthetic maintenance phase on a heated surgical platform. Core body temperature was measured using intraperitoneal RFID transponders, with concurrent monitoring of physiologic parameters and anesthesia recovery time. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Results: Heated induction rapidly achieved thermal stability and maintained normothermia during anesthesia induction. In contrast, unheated induction produced a rapid and clinically relevant decline in core body temperature. Mice undergoing unheated induction did not regain baseline temperature until several minutes into anesthetic maintenance and stabilized at approximately 1 °C below those receiving heated induction. Physiologic parameters and recovery times were comparable between conditions.

Conclusion: Heated anesthesia induction effectively prevents early peri-anesthetic hypothermia in mice without compromising physiologic stability. By addressing heat loss at its most vulnerable stage, this approach represents a practical refinement that improves animal welfare and reduces temperature-related experimental variability. Preventing hypothermia during induction should be considered a best-practice refinement in rodent anesthesia.



CO₂, CO, AND N₂ EUTHANASIA IN LABORATORY MICE: A MULTISYSTEM WELFARE ASSESSMENT

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Millions of laboratory mice are euthanized worldwide every year, most often with carbon dioxide (CO₂). However, CO₂ raises major animal welfare concerns, because it is aversive and induces strong respiratory distress, which may cause anxiety and pain.

Currently, insufficient data are available to assess and classify the impact of CO₂ and other gases proposed as alternatives. In this context, it is crucial to accurately determine when animals lose consciousness as the humaneness of an euthanasia method depends on the experience of pain or distress before loss of consciousness (LOC).

Here, we analyzed and compared the effect of CO₂, N₂, and CO euthanasia in female and male C57Bl/6N mice, one of the most widely used mouse strains. Mice were implanted with transmitters to record neurological and cardiovascular signals while assessing also respiration, behavior by video-tracking, and measuring biochemical stress parameters. This multi-modal assessment enabled us to determine the time points of loss of posture (LOP) and LOC.

CO₂ induced gasping and muscle fasciculations before and after LOP whereas CO and N₂ caused severe seizures and strong agitation, and delayed cessation of electrical brain activity. LOC, induced by the anesthetic properties of CO₂, was the fastest with a high-volume displacement rate of CO₂. Multi-modal assessment revealed that CO and N₂ led to a hypoxia-induced death that is less humane than with CO₂ euthanasia. CO and N₂ cannot be recommended for a more humane euthanasia of laboratory mice.

INHALANT ANESTHESIA BEFORE EUTHANASIA WITH CO₂ IN LABORATORY MICE: IMPROVEMENT OR CHANGE FOR THE WORSE?

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Carbon dioxide (CO₂) remains the most widely used method for euthanasia of laboratory mice. However, its use is associated with relevant animal welfare concerns, since it is aversive and induces respiratory distress, which may result in anxiety and pain. In our recent multisystem welfare assessment, we showed that CO₂ at a 30% volume displacement rate (VDR) produced the lowest overall distress burden, whereas CO₂ at 70% VDR induced faster loss of consciousness (LOC) but was associated with increased distress beforehand.

Building on these findings, the present study addresses a key refinement question: whether the welfare burden associated with CO₂ (30% VDR) can be further reduced by blunting the conscious phase through anesthetic induction. Although anesthesia before euthanasia has been proposed to limit aversion and distress, inhalational anesthetics itself can provoke respiratory irritation aversion and therefore distress, anxiety or even pain. It therefore remains unclear whether anesthetic LOC induction reduces the overall distress or merely shifts it to an earlier phase – compared to CO₂ only.

We investigated three commonly used anesthetic agents for induction prior to CO₂ (70% VDR) euthanasia: nitrous oxide (N₂O), isoflurane, and sevoflurane. N₂O provides anxiolytic and analgesic effects, but suppression of visible distress does not necessarily indicate LOC. Isoflurane and sevoflurane reliably induce unconsciousness but have been associated with excitation and respiratory irritation during induction. Using our multisystem framework, we quantified behavioral events, respiratory and cardiovascular responses, EEG/EMG endpoints, and biochemical stress indicators in female and male C57BL/6J and BALB/c mice.

This design not only complements our previous work by assessing sex- and strain-specific responses under CO₂ (30% VDR) alone, but also enables direct comparison with anesthetic-assisted protocols. By doing so, we determine whether anesthetic induction provides a measurable welfare benefit during euthanasia, and identify which strategies improve mouse welfare.

Keywords: Gas Euthanasia, Laboratory Mice, CO₂, Refinement, Distress

THE EVOLVING FRAMEWORK OF STANDARDS FOR ACCREDITATION OF ANIMAL CARE AND USE PROGRAMS

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A combination of legal requirements and other adopted standards forms the foundation of the AAALAC International (AAALAC) accreditation process. AAALAC has accredited more than 1,150 animal care and use programs in 52 countries and regions, including 131 programs in 22 European countries. Accredited programs must comply not only with applicable legislation but also with a defined set of international standards categorized according to their importance within the accreditation process.

At the top of the hierarchy of non-legal accreditation standards are the so-called *Primary Standards*. Two key Primary Standards are the *Guide for the Care and Use of Laboratory Animals* (the Guide) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). The Guide is currently undergoing revision, and the outcome is expected to be a substantially different type of document, with more frequent topic-specific updates. In contrast, although ETS 123 remains valid—since many housing and accommodation standards in Directive 2010/63/EU are based on Appendix A of ETS 123—the Council of Europe has no current plans to revise it. Consequently, ETS 123 may eventually become outdated in relation to other European legal instruments.

Within this evolving international context, and as a part of its most recent Strategic Plan, AAALAC is restructuring its accreditation standards framework. As an initial step, new Position Statements—representing the second level of standards after the Primary Standards—have been published on the 3Rs and Culture of Care, and the Position Statement on Veterinary Care has been revised. In addition, many existing Frequently Asked Questions are being transformed into a new category of standards known as *Guidance Statements*. The evolution and implementation of this updated accreditation standards framework will be described in detail.



CHARACTERIZATION OF A NEW AGGRESSIVENESS MODEL IN GÖTTINGEN MINIPIGS

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Background: The development of an aggressiveness model in pigs supports translational drug development by providing a large-animal system with human-relevant anatomy, physiology, and social behavior. Such a model enables evaluation of pharmacological interventions targeting social stress, impulsive aggression, and associated neuroendocrine responses under controlled yet ethologically relevant conditions. The resident–intruder paradigm offers a valuable approach to bridge the gap between rodent models and clinical application, improving prediction of efficacy and safety for compounds aimed at modulating social conflict and stress-related psychopathology.

Methods: Four uncastrated male Göttingen minipigs (residents) were single-housed. Two castrated male minipigs (intruders) were single-housed in a separate room. On day 1, each intruder was introduced sequentially to a resident for 10 minutes, and interactions were video-recorded. Dominant behavior, biting, lifting, and contact interactions were quantified. Forty minutes after interaction, animals underwent an open-field test. Blood and saliva samples were collected for stress biomarker analysis. Next, for model validation, the resident animals were treated with risperidone (1 mg/kg, p.o.) 2.5 hours prior to the exposure to the intruder.

Results: Residents immediately engaged intruders, displaying dominant behaviors such as mounting. The number of dominant behavior episodes was 28 ± 3 . Risperidone treatment reduced dominant behavior by approximately 50% ($p < 0.05$). In the open-field test, risperidone-treated animals showed reduced locomotor activity, as expected. In the approach test, treated animals exhibited prolonged latency to approach the entering researcher compared with vehicle-treated controls.

Conclusion: The resident–intruder paradigm represents a robust and translational model for studying aggression in Göttingen minipigs. Key endpoints include dominant behavior, locomotor activity, and approach behavior, supporting its utility for the evaluation of novel psychiatric drugs targeting aggression.

REFINING XENOTRANSPLANTATION RESEARCH: UTILIZING RETIRED SOWS FOR LUNG PERFUSION TO PROMOTE RESPONSIBLE ANIMAL USE

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Xenotransplantation, the transplantation of non-human organs into humans, is rapidly becoming clinical reality with initial FDA-approved trials. Nevertheless, further research is still needed. At Hannover Medical School – one of the largest transplant centers in Germany, a multidisciplinary team is working to bridge knowledge gaps. Utilizing complex genetically modified pigs as organ donors holds significant promise to reduce immune response in kidney transplantation. However, the lung remains particularly susceptible to rapid dysfunction upon contact with human blood. To address this challenge, ex vivo lung perfusion (EVLP) experiments are crucial enabling research on isolated organs. Organ retrieval and EVLP must be performed under sterile conditions, using established clinical protocols and equipment normally applied in human lung transplantation. Therefore, juvenile pigs are usually used as donors.

In order to maximize resource utilization and reduce the number of animals required, we aimed at including retired breeding sows in these analyses as refinement strategy. This presented logistical challenges regarding housing adaptation and surgical organ procurement. Specifically, the implementation of inhalational anesthesia with intubation and thoracotomy itself in large sows (350-400 kg) required careful consideration. A collaborative, multidisciplinary team – including scientists, animal caretakers, veterinarians and a cardiothoracic surgeon experienced with porcine thoracotomy – was assembled. Extensive planning, incorporating contingency measures and direct consultation with regulatory authorities guided the process. Procedures encompassed acclimation, pre-sedation, monitored transport, anesthesia including intubation, analgesia, thoracotomy and preparation of the lungs for EVLP with human blood. Managing intubation and adequate ventilation proved critical. All challenges were overcome and excellent lungs were used in EVLP experiments.

This study demonstrates the viable and ethical utilization of retired breeding sows for EVLP. The procedure proved comparable in feasibility and organ quality to younger swine, offering a valuable option for maximizing the use of genetically defined animals and promoting the refinement and reduction in xenotransplantation research.

References:

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EDUCATION and TRAINING FOR THE FUTURE: SKILLS, ETHICS & MENTORSHIP

ONE WELFARE: AN ATTEMPT TO LINK EVERYTHING

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The concept of One Welfare recognises the interconnectedness of animal welfare, human wellbeing, and environmental sustainability. Building upon the principles of One Health, One Welfare encourages a holistic and collaborative approach to challenges that arise at the interface of humans, animals, and the environment.

Within Laboratory Animal Science, the One Welfare approach offers an opportunity to strengthen dialogue and collaboration between researchers, veterinarians, animal care staff, policy makers, and society at large. By breaking down traditional disciplinary boundaries, it promotes a culture in which scientific quality, animal welfare, staff wellbeing, and societal responsibility are considered together rather than in isolation.

This keynote presentation will explore how One Welfare can contribute to a more sustainable and ethically responsible framework for the use of animals in science. It will reflect on the opportunities and challenges of integrating welfare, health, and environmental considerations, while striving to achieve the best possible balance between scientific progress, animal wellbeing, human wellbeing, and care for the environment.



THE EUROPEAN COLLEGE OF LABORATORY ANIMAL MEDICINE: TRAINING, CERTIFICATION, AND PROFESSIONAL IMPACT

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Veterinary specialization in Laboratory Animal Medicine plays a pivotal role in advancing animal welfare and scientific quality across biomedical research institutions. The European College of Laboratory Animal Medicine (ECLAM), recognized and governed by the European Board of Veterinary Specialisation (EBVS), establishes and maintains high professional standards within this field through rigorous education, examination, and continuing professional development.

This presentation provides an overview of ECLAM's structure, objectives, and certification pathways, outlining the requirements for residency training and board qualification within the College. Key aspects of the residency program—including eligibility criteria, training supervision, learning objectives, and assessment of competencies—will be discussed, emphasizing how residents acquire advanced expertise in laboratory animal science, legislation, ethics, and research management. The talk will also describe the ECLAM certifying examination and the evaluation process used to ensure fairness, consistency, and alignment with EBVS standards.

Beyond credentialing, ECLAM fosters a professional network that supports continuous learning and collaboration across academia, industry, and regulatory environments. Being a Diplomate of ECLAM not only provides access to expert communities and recognition as a European Specialist in Laboratory Animal Medicine but also offers opportunities to mentor colleagues across Europe, contribute to the training of the next generation of specialists, and further develop leadership skills and expertise in education. Membership benefits thus extend from career development and professional credibility to direct contributions to the refinement, reduction, and replacement (3Rs) of animal use in research.

By contextualizing ECLAM within the broader framework of European veterinary specialization, this talk aims to highlight its unique contribution to harmonizing education and professional excellence among laboratory animal veterinarians, ultimately advancing both scientific integrity and animal welfare across Europe.

References:

1. Home – European Specialists in Laboratory Animal Medicine <https://eclam.eu/>



DISENTANGLING POSTGRADUATE EDUCATION FOR LABORATORY ANIMAL VETERINARIANS

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Postgraduate education will transition a general veterinary practitioner into a Laboratory Animal Veterinarian who may also fulfil the responsibilities of a Designated Veterinarian or Named Veterinary Surgeon.

A number of formal training routes are available in Europe and the UK, including Board Diplomas, Professional Certificates, and Postgraduate Degrees. These programmes will provide structured learning on the key elements of laboratory animal medicine and lead to a formal qualification. However, these programmes vary, for example, in syllabus, requirements, intensity and duration. The ECLAM Diploma is considered the highest qualification. Continuous professional development courses are topical and useful for developing core competencies and maintaining knowledge and skills, and rely on the veterinarian to make informed choices regarding their learning needs and knowledge gaps. Mandated training as per a country's legislation e.g., EU Functions A, B, C, D (Article 23 of EU Directive 2010/63) will be covered elsewhere and therefore not by this presentation. Examples of postgraduate programmes will be used to compare and contrast their respective benefits aiding new entrants in their choice of learning routes.

References:

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2. Home – European Specialists in Laboratory Animal Medicine <https://eclam.eu/>



MAPPING LABORATORY ANIMAL VETERINARIANS' EDUCATION IN EUROPE AND 3RS INTEGRATION: A 2026 PERSPECTIVE

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Veterinary education in Europe operates within a well-established quality-assurance framework coordinated by the European Association of Establishments for Veterinary Education (EAEVE). Of the 123 veterinary education establishments (VEEs) registered in Europe, 96 are EAEVE members and 84 currently hold accredited or approved status. Central to this framework is the ESEVT Standard Operating Procedure (SOP) 2023, which defines 38 Day One Competences (D1Cs) required to ensure that graduates can practise veterinary medicine safely and effectively. Laboratory Animal Medicine and the principles of Replacement, Reduction and Refinement (3Rs) are explicitly embedded within these competences, notably through addressing scientific research methods and 3R implementation (D1C 1.2), and focusing on the biology, welfare and behavioural needs of laboratory-housed animals (D1C 2.3).

This study aims to provide an updated and comprehensive overview of how Laboratory Animal Medicine and the 3Rs are integrated into European veterinary curricula in 2026, with particular emphasis on the translation of regulatory expectations into clinical and practical training. A dual methodological approach is employed. First, a systematic review of Self-Evaluation Reports from EAEVE-member VEEs based on SOP2023 is conducted to assess the structure and learning outcomes related to LAM and integration of 3Rs. Second, a targeted questionnaire is distributed to VEEs to collect current data on dedicated LAM training tracks, exposure to the “Designated Veterinarian” role, and the implementation of the “never the first time on a live animal” principle.

The findings are expected to reveal substantial variability in Laboratory Animal Medicine education and delivery models, alongside a growing reliance on skills labs and alternative training methods to reduce early-stage animal use. By mapping current practices, this study provides a strategic overview to support further harmonisation of 3R-based education across Europe. The findings invite participants to share experiences and reflect on transferability to their contexts.



MAPPING DESIGNATED VETERINARIANS' EDUCATION AND TRAINING REQUIREMENTS AD IN EUROPE

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Directive 2010/63/EU (1) classifies all personnel involved in the use of animals for scientific purposes and related functions. Art. 23 of the Directive states that personnel responsible for animal care, performance of procedures (including culling) and designing procedures, must possess appropriate education and training. In addition, the Directive requires establishments to appoint a 'designated veterinarian with expertise in laboratory animal medicine, or a suitably qualified expert where more appropriate.' However, specific requirements for education, training, competence and continuous professional development are determined at the level of individual Member State.

The presentation aims to provide a comprehensive overview of education and training requirements across different European countries for veterinarians to cover the role of Designated Veterinarian. It will examine how these requirements shape the professional roles and responsibilities of Designated Veterinarians.

By highlighting similarities and differences between national approaches, the presentation seeks to contribute to a broader discussion on the mobility of veterinarians within Europe and the potential for greater harmonization and competency standards.

References:

1. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes



FEDERATION OF EUROPEAN LABORATORY ANIMAL ASSOCIATIONS WORKING GROUP RECOMMENDATIONS ON “TRAINING FOR REPLACEMENT”

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Replacement is embedded in Directive 2010/63/EU as the long-term objective for scientific research involving animals. Despite rapid advances in non-animal technologies (NATs) and new approach methodologies (NAMs), the systematic implementation of Replacement remains limited and inconsistent across institutions, disciplines and career stages. Researchers, animal welfare bodies, ethics committees and project reviewers frequently report insufficient expertise in identifying, evaluating and adopting non-animal methods. Existing education and training resources are fragmented, often outdated, and tend to prioritise reduction and refinement rather than early and explicit consideration of replacing animal use altogether. Guidance on how to search for, assess and transparently document alternatives is similarly limited, constraining progress toward human-relevant research practices.

To address these gaps, FELASA (Federation of European Laboratory Animal Associations) Working Group on “Training for Replacement” developed a standardised, competency-based syllabus to support harmonised, high-quality education in replacement strategies. The syllabus defines core learning outcomes for participants, aligned learning objectives for tutors, and practical guidance for course structure, delivery, assessment and evaluation. It is organised into five interconnected sections: (1) introduction to replacement and the scientific, ethical and regulatory foundations of the 3Rs; (2) development and reframing of research questions to enable non-animal approaches; (3) identification of information sources and effective searching strategies, including bibliographic databases, grey literature and expert networks; (4) critical assessment of search results for scientific relevance, credibility and regulatory suitability; and (5) a practical, skills-based short course introducing selected NAMs in specific research contexts.

The framework is intended for a broad, multidisciplinary audience. This syllabus aims to strengthen confidence and competence in non-animal approaches, accelerate adoption of human-relevant methodologies, and support progress toward the long-term goal of phasing out animal use in research and testing.



DESIGNING FOR WELFARE: FACILITIES, TECHNOLOGY & SUSTAINABILITY

ACUTE MORTALITY IN IMMUNOCOMPROMISED MICE LINKED TO *PAENICLOSTRIDIUM SORDELLII*-INDUCED CYTOLYSIN-MEDIATED SEPTICEMIA

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Immunocompromised mouse strains, such as NOD.Cg-Prkdcscid-Il2rgtm1Wjl/SzJ (NSG) and NOD.Cg-Rag1tm1Mom-Il2rgtm1Wjl/SzJ (NRG), are invaluable in biomedical research. However, these strains pose significant health management challenges due to their susceptibility to infections with opportunistic bacteria and common pathogens. While breeding in strict hygienic barrier units is a prerequisite for maintaining the health integrity, these mice remain at risk after transportation to experimental barriers for research procedures.

We recently observed severe diarrhea and sudden deaths in three cohorts of NSG and NRG mice housed in an individually ventilated cage (IVC) system within an experimental barrier unit. Occasionally individual mice of immunocompetent, genetically engineered mouse strains showed also clinical symptoms. Both genders and all age groups were affected. Gross pathology revealed edematous to hemorrhagic intestinal inflammation, while histopathological analysis showed massive cell lysis of the intestinal epithelium. Intestinal content from affected mice was transferred into healthy NSG recipients via oral gavage, inducing a similar disease phenotype within 4-6 days, suggesting an infectious etiology.

Extensive microbiological and virological diagnostics ruled out an infection with common murine pathogens. However, *Paeniclostridium sordellii* was isolated from the blood, livers, kidneys, and intestinal contents of 13 out of 23 affected mice. PCR analysis revealed that the isolated strains consistently expressed a cholesterol-dependent cytolysin, indicating that fatal septicemia resulted from toxin-induced damage to the intestinal epithelium and subsequent intestinal hyperpermeability.

These findings highlight *Paeniclostridium sordellii* and other *Clostridiaceae* as emerging pathogens causing infectious diseases in immunocompromised mouse colonies, underscoring the need for enhanced surveillance and biosecurity measures in such high-risk populations.

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HEALTH SCREENING: GOING ANIMAL-FREE

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In support of the 3Rs and best practice in laboratory animal veterinary medicine, the University of Aberdeen reviewed its use of live animals for routine rodent health monitoring. The primary aim was to assess whether animal-free screening methods could replace sentinel animals while maintaining, or improving, the quality of diagnostic information required for veterinary oversight, colony health management and regulatory reporting.

Following evaluation of several alternative approaches, an adapted shake-cage method using a molecular sampling system (Ghost Sentinel®, FERA Science) was selected for in-house trials. This approach allows direct environmental soiled bedding sampling from cages, providing material suitable for comprehensive pathogen detection without the use of live sentinel animals.

Two comparative trials were conducted, comparing the existing soiled bedding sentinel programme with the Ghost Sentinel® method. Samples were collected from both open cages and individually ventilated cages (IVCs) and analysed by two independent diagnostic laboratories. An extensive panel of viral, bacterial and parasitic agents was tested in line with FELASA recommendations.

All agents previously identified through sentinel monitoring were also detected using the Ghost Sentinel® system. Importantly, direct molecular sampling showed greater consistency and sensitivity, enabling earlier and more reliable detection of pathogens. One agent not previously reported using sentinel animals was also identified. These findings have clear implications for veterinary risk assessment, outbreak management, health status reporting and decision-making regarding biosecurity and colony management.

This presentation will discuss the validation process, interpretation of results and subsequent implementation. The data demonstrate that animal-free health monitoring can enhance veterinary diagnostic confidence while supporting the 3Rs, particularly replacement. This approach has been successfully implemented at the University of Aberdeen since January 2024.



WHEN WELFARE HIDES IN PLAIN SIGHT: LATENT STATE MODELS FOR SEVERITY MONITORING

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Introduction: Current severity assessment in laboratory animal research relies on static thresholds or subjective scores that may fail to capture the dynamic nature of animal welfare¹.

Aims: We propose a Hidden Markov Model (HMM)² a probabilistic framework, that infers an animal's true welfare condition from continuous observations over time, enabling dynamic, uncertainty-aware monitoring.

Materials and Methods: Using the Relative Severity Assessment (RELSA) score³ as input, which integrates body weight change, heart rate, heart rate variability, activity, and core temperature, the HMM estimates the likelihood of being in one of three welfare states (Well, Transition, Impaired). These estimates are converted into an intuitive traffic-light system with continuous risk curves and alarms for humane endpoints. We validated the framework using computer simulations (B = 50 replications, 300 animals each) across three scenarios and applied it to published dextran sodium sulfate (DSS)-colitis and transmitter-implantation mouse data.

Results: In controlled simulations, classification accuracy exceeded 90%, and confidence estimates were well-calibrated. Under high-noise conditions with overlapping state distributions, accuracy dropped to 46%, confirming that the model signals uncertainty rather than overconfident misclassifications. Application to experimental data revealed biologically plausible severity patterns: risk curves rose during disease flares and post-surgical periods, while controls remained predominantly in the Well state. Chi-squared analysis confirmed significant differences in state distributions between treatment groups (DSS: $\chi^2 = 38.4$; transmitter: $\chi^2 = 54.9$; both $p < 0.001$). Sensitivity analysis showed alarms remained stable across varying thresholds.

Conclusion: This framework enables real-time severity monitoring at the individual-animal level, providing probability-based risk curves and automated alarms to support timely intervention decisions. The approach extends from monitoring individual animals to laying the groundwork for broader welfare oversight within automated home-cage systems, providing a consistent basis for large-scale, ongoing welfare evaluation that advances the principles underpinning the 3R concept.

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LISTEN TO YOUR RABBITS WHEN DESIGNING FLOOR PENS FOR SOCIAL HOUSING NAIVE RABBITS

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In 2024, Inotiv UK created a new facility for social housing of New Zealand White rabbits. Prior to creating the new facility, our practice, in our breeding barrier, was to wean rabbits at five weeks of age into pairs or trios and maintain them in cage housing until the point of sale.

The new facility allowed rabbits to be housed in floor pens, with rabbits weaned directly into groups of forty rabbits. The pens were designed to allow rabbits to interact with each other across a 60,000 cm² 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 demonstrate 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.

In the two years since we began using the floor pens, we have seen both a positive effect on the rabbits and feedback from customers who have noted a calmer behaviour in the animals they have purchased.

From the original pen design to the current pen set-up, it has been a long dynamic process with continuous changes and improvements to meet the rabbits' needs, learning from the rabbits their likes and dislikes. Examples of the changes include the pen material, pen height, food hoppers, hay nets, enrichment, and increased platform size and huts.

INTEGRATED ENVIRONMENTAL AND BIOLOGICAL MATERIAL MONITORING TO ADVANCE ETHICAL PRINCIPLES IN RESEARCH RODENT COLONIES

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Traditional rodent health monitoring using soiled bedding sentinels (SBS) suffers from low sensitivity, inconsistent performance, and animal welfare concerns. Strong supporting evidence for SBS effectiveness exists for only a limited range of pathogens, making it increasingly insufficient for today's laboratory animal programs. In contrast, Environmental Health Monitoring (EHM), including exhaust dust testing, sentinel-free soiled bedding, and direct colony sampling, provides a more sensitive, consistent, and efficient monitoring strategy. EHM is supported by a broader and more robust peer-reviewed evidence base and delivers clear operational advantages, such as reduced labor and cost, while eliminating the need for sentinel animals.

Simultaneously, the expanding use of biological materials, particularly patient-derived xenografts and diverse cell lines, has increased the risk of introducing microbial and viral contaminants into research animal colonies. When insufficiently monitored, these contaminants can compromise animal health, disrupt experimental outcomes, and undermine data validity and reproducibility. Such failures not only waste animals and resources but also threaten the integrity of the scientific work itself.

Integrating EHM with rigorous screening of biological materials represents a practical and impactful application of the 3Rs principles. Refinement is achieved through more accurate and proactive health monitoring; reduction results from preventing study failures driven by undetected infections; and replacement occurs through the elimination of sentinel animals. Together, these complementary strategies reinforce animal welfare, enhance scientific rigor, and strengthen institutional research integrity.



COMMUNICATION & TRUST: GLOBAL PERSPECTIVES FOR SCIENCE, SOCIETY

COMMUNICATING THE 3RS TO YOUNG PEOPLE

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The use of animals in research is controversial and often emotive. Young people develop values and opinions from parents, teachers and peers during adolescence when worldviews are actively formed and challenged. School curricula in many countries include studies in philosophy and ethics. Animal research is frequently discussed here because of its contentious nature. Anti-vivisection organisations often provide educational material for schools, however, input from those working in laboratory animal science is usually absent, which may result in unbalanced information.

Our institution strongly supports openness and transparency, as these are essential for building public trust. We believe opinions should be informed by accurate and sufficient information, therefore we have developed links with local schools and public institutions to inform teachers, young people and families about the animals we use in research and how we apply the 3Rs.

Our outreach activities include a science centre event for primary-school children (5-11), featuring interactive activities to help them understand how rodents perceive the world. These include following an ultraviolet trail to simulate urine marking and completing a jigsaw while wearing goggles smeared with petroleum jelly to demonstrate rodents reduced visual acuity. They also observe live wax-moth larvae under the microscope as an example non-vertebrate model.

We deliver workshops for secondary-school pupils (11-17), encouraging discussion around species choice, limitations of non-animal alternatives and refinement of housing and procedures. We run sessions for work-experience students aged 16+, covering the ethics of animal research, including the Mouse Exchange¹ as an interactive activity, and provide tours of our animal facilities. We assess students' views before and after activities using anonymous voting software. We consistently observe a shift towards increased support for animal research and reduced concerns following outreach events.

We are currently working with a Dutch institute to connect UK and Dutch students for discussions on animal research.

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ANIMAL RESEARCH, NAMS AND PUBLIC TRUST: WHERE ARE WE HEADING?

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New Approach Methodologies (NAMs) are rapidly advancing and gaining scientific, regulatory, and political attention. Organoids, organs-on-chips, AI, in silico approaches, and other human-relevant models are ready to provide valuable insights, and their development should already be seen as part of the 3R strategic implementation. However, the current debate often presents NAMs as a near-term replacement for animal research, neglecting their limitations and the ongoing need for animal studies in crucial biomedical areas.

This presentation will argue for a balanced approach. NAMs should be seen, by scientists and society alike, as part of a broader research toolbox, supporting replacement where possible and even answering some initial biological questions that once required animal research, making animal use much more targeted. Nevertheless, contrary to widespread misinformation, they cannot fully replace animal studies: they are not able to either reproduce whole-body responses or complex interactions among organ systems, which are essential in many fields such as basic research in immunology, neurosciences, endocrinology, infectious diseases, nor to validate translational research and safety assessment before clinical trials.

Misinformation shapes public and policy narratives. The talk will address distortions such as the “95% failure rate” claim and contrast them with public-ready evidence showing substantial concordance between animal and clinical findings. It will also include recent policy trends that risk reducing the 3Rs to replacement alone, potentially compromising responsible science and animal welfare if decisions go beyond scientific evidence.

The research sector must respond with clarity and openness. EARA is a global advocacy and communications association that aims to enhance public understanding of the continued necessity of animal research, the progress in welfare and the 3Rs, and the complementary nature of NAMs and animal research. We support our members in transparent communication and position it as the only way to proactively combat public misinformation about animal research.

Keywords: Animal research, NAMs, 3Rs, transparency, openness, science communication, policy, animal welfare, Europe+



A STEP FURTHER TOWARDS A TRANSPARENCY AGREEMENT IN GREECE

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National Transparency Agreements on animal research are important tools for promoting openness and strengthening public trust in the biomedical sector. To date, ten countries have established such agreements under the guidance of the European Animal Research Association. These initiatives bring together researchers and communication professionals to reduce barriers and encourage proactive communication about the use of animals in research.

We conducted a nationwide survey among public and private institutions in Greece that use animals for scientific purposes, in order to assess their motivation to participate in a Greek Transparency Agreement (TA). Facility managers were asked whether they would be willing to support the four core commitments of the TA, which focus on enhancing both internal and external communication, improving openness, and promoting the exchange of experiences and best practices.

The survey revealed strong positive feedback and a clear willingness to move towards the establishment of a national agreement. These preliminary results were presented at the FELASA 2025 Congress and at the EARA meeting in Berlin in 2025. Building on this momentum, the Hellenic Society for Biomedical and Laboratory Animal Science (HSBLAS) has joined this initiative, and discussions have begun regarding the practical steps required for the development of a Greek TA. In February, the first joint meeting was convened, bringing together representatives from EARA, HSBLAS, and Greek animal facilities. At least five facilities have already committed to participating in the initial implementation phase.

At the CELASC meeting, we will present the progress achieved towards the establishment of a Transparency Agreement in Greece, along with additional data from our survey regarding the species used in research and their relative frequency. These findings represent an important step towards fostering a culture of openness and coordinated communication at the national level.



LINKING PLANNING, PRACTICE, AND REPORTING: THE EQIPD QUALITY SYSTEM AS A FRAMEWORK FOR HIGH-QUALITY ANIMAL RESEARCH

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Planning as the foundation for quality animal research

High-quality animal research begins with robust planning, supported by early and structured dialogue between research groups and animal facilities. Such engagement is essential for aligning scientific objectives with animal welfare considerations, refining experimental design, and anticipating operational risks. Guidance such as PREPARE² provides a strong foundation by promoting shared responsibility and transparency at the outset of animal studies. However, planning alone cannot ensure consistent quality throughout study execution and reporting.

The EQIPD Quality System for research practice

The EQIPD Quality System¹ was developed as a flexible framework to assure quality during research practice, where many critical decisions influencing scientific rigor and animal welfare are made. It embeds structured yet proportionate processes for study conduct, documentation, data integrity, and risk-based decision-making. By translating well-prepared study plans into traceable everyday practices, the EQIPD Quality System supports consistency, scientific robustness, and feasibility in academic and pre-clinical animal research environments. In doing so, the EQIPD Quality System has been implemented across diverse laboratory types and research settings worldwide, demonstrating its adaptability and relevance in different institutional and cultural contexts.

Enabling transparent reporting and long-term impact

Complete and transparent reporting is essential to maximize the scientific value of animal studies and avoid unnecessary duplication. The EQIPD Quality System facilitates alignment with ARRIVE Guidelines³ by ensuring that key methodological and analytical details are systematically captured during the study rather than reconstructed retrospectively.

By linking planning, practice, and reporting, the EQIPD Quality System strengthens rigor, supports the 3Rs, and provides laboratory animal science with a coherent framework for sustainable, high-quality research.

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2. PREPARE <https://norecopa.no/PREPARE>
3. ARRIVE <https://arriveguidelines.org/>

HUNGARIAN PLATFORM OF INSTITUTIONAL ANIMAL WELFARE BODIES: MISSION, STRUCTURE, AND NATIONAL IMPACT

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The Hungarian Platform of Institutional Animal Welfare Bodies (HUPAWB) is a nationwide umbrella organization that was established in 2022 with the aim of serving academic and industrial institutional AWBs to harmonize and improve laboratory animal science across the country. HUPAWB enables the exchange of experiences and best practices between member AWBs, fostering mutual learning and methodological development. As such, HUPAWB members have created a unified operating framework, defined harmonised guidelines at the national level, and took the leading role in promoting modern laboratory animal science emphasizing the 3Rs and new alternative methods.

Currently HUPAWB has around one hundred members who are appointed representatives of the local AWBs of their institutions and are directly involved in the first-stage ethical review process of the (ethical) project licence applications involving procedures on laboratory animals. Members may also act as advisors for researchers throughout the application process. The platform regularly holds online meetings and an annual conference in person. Recently, a new website has been launched (www.omab.hu), which serves to inform scientists and technical staff on advancements in the field and support advocacy and communication on animal science and welfare. In this context, members actively participate in the nationwide *Researchers' Night* program series and in similar events. In 2024, the HUPAWB joined the European Network of National Networks of Animal Welfare Bodies (ENAWB), to strengthen our national and international partnerships, expand outreach and communication especially to students of high-schools and universities and to the general public.



THE POLISH NETWORK OF ANIMAL WELFARE BODIES: EARLY EXPERIENCE AND FUTURE DIRECTIONS

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Animal Welfare Bodies (AWBs) are entrusted with a broad range of responsibilities related to the welfare of animals used for scientific purposes, including advisory and oversight functions. In practice, their effective fulfilment is often challenged by a high workload of AWB members and the absence of formal institutional recognition for animal-welfare-related duties. As a result, AWB structures and practices vary significantly across Polish institutions.

In April 2025, 10 years after the implementation of Directive 2010/63/EU in Poland, the Polish Network of Animal Welfare Bodies (Polska Sieć Zespołów do spraw Dobrostanu Zwierząt) was established as an informal, bottom-up initiative to strengthen AWB performance nationwide and facilitate integration into European structures. To date, more than 40 AWBs – approximately one third of those operating in Poland – have joined the network voluntarily.

The network's core activities focus on facilitating the exchange of experience and good practices, harmonising operational procedures, providing mutual scientific, ethical, and regulatory support, and acting as a coordinated platform for dialogue with national authorities. As an initial step, a detailed survey was conducted among member AWBs to assess current practices, identify effective organisational models, and determine key barriers to optimal functioning. The survey findings will inform the establishment of thematic working groups tasked with developing practical tools to support AWBs in their statutory responsibilities. These activities will culminate in the first hands-on workshop for AWB members, organised in cooperation with the Polish Laboratory Animal Science Association (PolLASA) in September 2026.

Shortly after its formation, the Polish network joined the European Network of National Networks of Animal Welfare Bodies (ENAWB). Participation in the ENAWB provides a framework for strengthening national systems, harmonising practices across the EU, and promoting the 3Rs through cooperation.



EUROPEAN NETWORK OF NATIONAL ANIMAL WELFARE BODY NETWORKS (ENAWB): SUPPORTING COLLABORATION AND CULTURE OF CARE IN EUROPE

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Animal Welfare Bodies (AWBs), mandated by Directive 2010/63/EU, play a key role in animal welfare oversight, implementation of the 3Rs (Replacement, Reduction and Refinement), and promotion of a strong Culture of Care within establishments using animals for scientific purposes. Experience from several European countries shows that structured national networks of AWBs strengthen these functions by enabling exchange of best practices, improving internal processes, supporting staff wellbeing, and contributing to scientific quality. However, national AWB networks are not yet established in many countries, limiting opportunities for collaboration and shared learning.

At the 2022 FELASA Congress, a dedicated session highlighted the value of national AWB networks in fostering a Culture of Care across Europe. Representatives of the existing networks subsequently initiated the European Network of National Animal Welfare Body Networks (ENAWB) as an informal umbrella organisation to facilitate bottom-up, voluntary collaboration among national AWB networks. Currently, thirteen countries are represented in the ENAWB.

ENAWB, with the support of the European Commission, aims to promote the establishment of national AWB networks where they do not yet exist, support the further development of established networks, and encourage greater consistency in AWB practices across Europe. The network provides a platform for exchange of information and experiences between AWBs, sharing of good practices and innovations, and joint reflection on common challenges related to animal welfare, the 3Rs, scientific quality, and staff wellbeing. ENAWB also contributes to the dialogue with animal welfare professionals, Competent Authorities, National Committees, and relevant European organisations.

By strengthening collaboration between AWBs at national and European levels, ENAWB supports effective implementation of Directive 2010/63/EU and reinforces a shared, sustainable Culture of Care across European animal research establishments, ultimately serving both animal welfare and scientific integrity.

ENAWB's website is currently hosted by Norecopa: <https://norecopa.no/ENAWB>



3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

LIP SERVICE, OR REAL IMPROVEMENT? USING GUIDELINES INTERACTIVELY ON THE PATH TO BETTER SCIENCE

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Are scientists merely paying lip service to funders and journals when they cite guidelines for planning, conducting and reporting animal studies? And if so, can we make them more aware of (and more enthusiastic for) the immense amount of guidance that the Laboratory Animal Science community can now offer?

A casual glance at publications describing animal research shows that the existence of guidelines for planning, conducting and reporting animal studies is, unfortunately, no guarantee of research quality. How can we encourage scientists to look upon these resources as tools for better science, rather than buzz words to mention, or hurdles to pass, on the road to funding and publication?

Norecopa has spent considerable resources on developing generic guidelines for planning studies which appear to involve animal use, and then directing scientists to more specific guidance for their individual needs.

This presentation will describe how the PREPARE guidelines can be used from day 1 of planning to implement all three Rs (Replacement, Reduction, Refinement), in such a way that scientists realise the value of paying more than lip service to these principles.

The presentation will also present novel ways in which Artificial Intelligence can be combined with recognised resources within Laboratory Animal Science to raise scientists' awareness of their need for improved quality control, and to provide them with the guidance they need to achieve this.

References:

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EFFECTS OF REPEATED VOLATILE ANESTHESIA IN LABORATORY MICE AND RATS

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Volatile anesthetic gases are widely used in *in vivo* research due to their rapid induction, controllability, and favorable safety profiles. Isoflurane is the most commonly used agent, yet its pungent odor has raised concerns regarding aversion and discomfort, particularly during repeated exposures. Sevoflurane, which lacks a pungent smell, has been proposed as a potential alternative, although its suitability for repeated anesthesia in mice and rats remains insufficiently characterized.

In this study, we investigated the effects of repeated exposure to isoflurane and sevoflurane, in laboratory mice and rats. Male and female individuals of both species (BALB/c, C57BL/6J, Wistar and Sprague Dawley) aged ten weeks were exposed three times to the same anesthetic gas. Aversion and respiratory parameters were assessed using whole body plethysmography during baseline, induction, maintenance, and recovery phases. Loss of posture was used as an indicator of anesthetic depth. Blood samples were collected after each exposure, and animals were euthanized after the final session for tissue and organ analyses.

Our preliminary results in mice showed no sex related differences in time to loss of posture. Strain dependent differences were observed during the first exposure to isoflurane and sevoflurane, but these differences were no longer present during subsequent exposures.

Our upcoming findings could highlight important strain dependent responses to repetitive volatile anesthetics exposure and their potential distress induced in mice and rats. Ongoing analyses of behavioral, respiratory and stress related parameters will further inform potential refinement strategies for repeated volatile anesthesia in laboratory mice and rats.



DEVELOPMENT OF MONITORING SHEETS FOR SHEEP AND PIGS UNDERGOING CARDIAC SURGERY

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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.



A RETROSPECTIVE: SEVERITY ASSESSMENT IN MICE USING BODY WEIGHT AND VOLUNTARY WHEEL RUNNING

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Since the entry into force of Directive 2010/63/EU, the legal classification of procedure severity has become mandatory, both for prospective project authorization and for retrospective reporting of the actual severity experienced by animals. Although Annex VIII of the Directive provides examples of expected severity categories, it offers limited guidance on how these were derived. To enable an evidence-based comparison between prospective and actual severity, this study retrospectively analyzed body weight and voluntary wheel running (VWR) data from several mouse models.

Data from approximately 300 C57BL/6 sub-strain mice subjected to interventions such as blood sampling, surgery, and disease models (pancreatic cancer, colitis) were analyzed. Severity was assessed using clinical score sheets including changes in body weight and VWR monitoring. An algorithm (RELSA) was then applied for severity quantification and comparison with both the prospective severity classification from the authorization process and the actually reported severity.

The retrospective analysis revealed a more differentiated classification of procedures than provided by the legal framework. As expected, mice that were only handled exhibited the lowest severity scores, whereas animals subjected to invasive surgeries showed the highest scores. Although all reviewed procedures had been prospectively classified as “moderate” according to official requirements, the analysis identified transitions between low and moderate severity that are not explicitly addressed in current guidelines. This transition zone is particularly relevant for timely definitions of humane endpoints and discontinuation criteria. Although the prospectively assumed maximum severity was confirmed, this was not consistent across all animals.

Overall, these findings suggest that current legal severity classifications may not fully reflect the actual severity experienced by animals. The integration of objective behavioral readouts and algorithm-based tools into routine severity assessment could improve the accuracy of project evaluation, support refinement strategies, and strengthen the practical implementation of the 3Rs.

SHAPING LAS EDUCATION – FELASA'S COMMITMENT TO THE 3RS

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FELASA (Federation of European Laboratory Animal Science Associations) accreditation is a key driver in improving the quality, ethical integrity, and consistency of laboratory animal science training across Europe and beyond. It ensures that courses and training programs in laboratory animal sciences align with Directive 2010/63/EU and the latest expert working group recommendations, which require all personnel involved in animal research to be properly trained.

FELASA has updated its accreditation framework to strengthen the importance of constructive alignment between learning outcomes, teaching methods, and assessment, while promoting high ethical standards and robust internal and external quality-assurance processes. These measures foster transparency, mutual confidence, and the seamless recognition of qualifications across institutions. The new approach also underscores how accreditation supports the 3Rs (Replacement, Reduction, Refinement). It does so by encouraging tiered training pathways, realistic and ethically responsible learning environments, and structured assessments that help learners build and demonstrate essential skills.



FELASA WORKING GROUP RECOMMENDATIONS FOR THE HUMANE KILLING OF LABORATORY FISH

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The Directive 2010/63/EU lists some methods to kill laboratory fish and was amended to add hypothermic shock, in specific conditions, for zebrafish only. Scientific projects may require the use of other techniques to achieve their scientific needs. However, environmental impact, health and risks, developmental stages and impact on animal welfare must also be considered when choosing the method of euthanasia. Following the first technique to kill fish, to comply with the directive, it is essential to perform a second method to complete and confirm the death of the animal. The FELASA Working Group on methods of humane killing of laboratory fish published their recommendations, reflecting on what would constitute an acceptable death, proposing comparisons between methods, and describing practical approaches to comply and refine according to the species, developmental stages, and context. Here, we will review experimental data pillar to these recommendations (such as efficacy and aversion of anaesthetics, hypothermic shock, electrical stunning, and concussion – percussive blow to the head), compare the methods, and focus on common and useful considerations for zebrafish according to developmental stages. Noticeably, we will differentiate between the termination of a colony when samples are not required, versus examples of killing a fish to obtain and culture biological material.

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3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

THE MI-RAT, A NOVEL MURINE MODEL FOR THE STUDY OF OSTEOARTHRITIS

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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 OA 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 translational model. The surgical models that are the most widely 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 changes¹. Subsequent studies using this model confirmed the influence of sexual hormones, particularly estrogen, in pain control, and the necessity to work on ovariectomized females. 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 homogeneous structural alterations, and corresponding functional and molecular pain signals², including epigenetics². Moreover, the application of quantitative sensory testing to MI-RAT offers a non-invasive assessment of facilitatory/inhibitory pain processes³. In conclusion, these recent developments and validation of the murine MI-RAT enlarged our perspectives in a refined translational osteoarthritis model.

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BEFORE THE MOUSE, THE MODEL: HYPER-REALISTIC SIMULATION AS A RODENT TRAINING REFINEMENT STRATEGY

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Introduction: Training in rodent biometrics and surgical techniques has traditionally relied on live animals or cadavers, raising ethical concerns and limiting opportunities for repeated, standardized practice. This approach conflicts with the principles of the 3Rs and can expose both animals and trainees to avoidable risk during early skill acquisition.

Aims: To evaluate hyper-realistic simulation-based training as a refinement strategy for rodent biometrics and surgical education, to improve competency while reducing reliance on live animals.

Materials and Methods: A series of anatomically accurate, task-specific rodent simulators was designed and validated to support training in commonly performed biometrics and surgical procedures. Models were developed using a combination of 3D printing, silicone molding, and soft plastic casting to achieve high anatomical fidelity, durability, and low cost. The modular design allows task deconstruction, repeated practice, and progressive skill development. Simulators were integrated into a structured training program through learning objectives and disseminated through hands-on workshops in Europe, Latin America, and the US.

Results: To date, more than 500 technicians, researchers, and veterinary professionals have been trained using these models. Participants consistently reported increased confidence and preparedness prior to performing procedures in animals. Quantitative assessments conducted for selected models demonstrated improvements in procedural accuracy, reduced error rates, and improved task efficiency following simulator-based training. Qualitative feedback highlighted the perceived anatomical realism of the models and the value of a safe, repeatable learning environment that supports early skill development without animal use.

Conclusion: Hyper-realistic rodent simulators represent an effective refinement for training in rodent biometrics and surgery, reducing and in some cases replacing the need for live animals during early training stages. Their implementation promotes ethical practice, standardizes instruction, and supports global dissemination of best practices aligned with the 3Rs. Simulation-based training should be considered a core component of modern, competency-based laboratory animal science education.

ULTRASOUND GUIDED SERRATUS PLANE BLOCK IN PIGS: ANATOMICAL STUDY AND DESCRIPTION OF THE TECHNIQUE

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Experimental pigs undergoing thoracic surgery require a multimodal analgesic approach. The serratus plane (SP) block is a fascial anaesthetic technique that, in humans and in dogs¹, provides effective analgesia of the chest wall².

The aim of this study was to evaluate the normal and the ultrasound anatomy of the serratus plane in pigs, and to describe the technique for performing the SP block.

Seven commercial hybrid pig cadavers (mean weight 35.6kg) were included. An anatomical study was first performed in one cadaver to define the anatomical features and to identify the anatomical landmarks. In the six remaining cadavers the ultrasonographic examination and the injection of the staining solution were performed in one hemithorax. With the animals in lateral recumbency, a linear probe was positioned on a line parallel to the spine and at the level of the scapulo-humeral joint as a reference.

The deep SP was then identified as a hyperechoic line above the external intercostal muscles; ropivacaine (0.25%) 0.3 ml/kg diluted with methylene blue (3:1 ratio) was injected at the 6th and 9th intercostal spaces. The anatomical dissection highlighted the distribution of the injectate between the intercostal muscles, and both beneath and superficial to the SP. In two cases the spread of the dye into the pleural space was observed.

The described technique was feasible in pigs, and the spread of the solution suggests that it might provide thoracic wall analgesia through sensory block of the lateral cutaneous branches of the thoracic spinal nerves and the long thoracic nerve as described in other species¹. Strict adherence to the reference landmarks is pivotal for the success of the block. Further studies are needed to understand the in vivo effectiveness of this technique for thoracic analgesia, thereby providing a significant refinement for perioperative pain management in pigs.

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NEW APPROACH METHODOLOGIES (NAMs): A THREAT OR A CHALLENGE FOR LABORATORY ANIMAL VETERINARIANS (LAVs)

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NAMs are an umbrella term for any technology or approach that can provide information on chemical hazards or biological effects without using animals, or by reducing their number or refining experimental procedures. Cell & tissue models, organoids, organs-on-chips, in silico models, and human-based data may be used to obtain data that are more human-relevant, faster, and often cheaper, while improving animal welfare.

The rise of NAMs presents several existential and practical challenges for the LAV community, which could be considered as a professional threat. These are linked to: (a) Career Displacement: The most obvious fear is the reduction in the "patient" population. (b) Skill Gap Re-tooling: Many LAVs spent a decade mastering animal surgery, anesthesia, and colony health. NAMs require knowledge and skills not traditionally taught in veterinary school. (c) Funding Shifts: Research grants are increasingly earmarked for "animal-free" innovation. (d) Identity Crisis: There is a deeply rooted professional identity in being the "voice for the animals" within a research institution. If there are no animals, the veterinarian must redefine their value proposition to the scientific team. Although this is a hard truth, there is also another option, much more promising for LAVs.

New Approach Methodologies (NAMs) can be considered as an invitation for LAVs to play a broader role as ethical decision-makers, helping determine when animal use is truly necessary and advising on 3Rs implementation. They can also serve as translational science experts, interpreting NAM data in the context of whole-organism physiology and guiding study design to maximize welfare and scientific rigor. Furthermore, NAMs open new career avenues in organoid research, computational biology, regulatory science, and 3Rs leadership. Rather than being replaced, veterinarians may become the main players in integrating NAMs responsibly, ensuring both ethical and high-quality biomedical research.



ANAESTHETIC PROTOCOL SELECTION IN LABORATORY ANIMAL RESEARCH: ALIGNING ANIMAL WELFARE AND SCIENTIFIC REQUIREMENTS

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The selection of anaesthetic and analgesic protocols in laboratory animal science is a major refinement opportunity, as anaesthetic agents exert wide-ranging effects on physiology that can influence both animal welfare and the validity of scientific outcomes. Across commonly used laboratory species, anaesthesia should therefore be regarded as a biologically active component of the experimental system rather than a neutral technical prerequisite.

Different categories of scientific procedures impose distinct physiological constraints that should guide protocol selection. In studies involving the central nervous system, including neurosurgery, neurophysiology, and imaging, anaesthetic depth and agent choice can markedly affect cerebral blood flow, neurovascular coupling, and neuronal network activity, with potential consequences for data interpretation if these effects are not carefully controlled. Similarly, in cardiovascular and cardiorespiratory research, many routinely used anaesthetic agents alter heart rate, vascular tone, blood pressure, and respiratory function in a dose and species-dependent manner, risking confounding of primary endpoints if haemodynamic stability is not prioritised. Anaesthetic choice is also increasingly recognised as relevant to immunology, inflammation, and infectious disease models. Both volatile and injectable agents have been shown to modulate innate and adaptive immune responses, influence cytokine release, and interact with peri-procedural stress pathways. Where immune parameters constitute key experimental outcomes, the consistency of anaesthetic exposure and the explicit justification of the protocol choice are therefore essential.

Adopting a procedure-led approach to anaesthetic selection, informed by the dominant biological system under investigation, supports refinement by improving welfare during induction, maintenance, and recovery, while simultaneously enhancing scientific robustness and reproducibility. Aligning anaesthetic practice with the specific physiological vulnerabilities of each experimental model provides a pragmatic framework for advancing both ethical and scientific standards in laboratory animal research



REPEATED ISOFLURANE ANAESTHESIA AS A WELFARE AND SCIENTIFIC VARIABLE IN MICE

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Introduction: General anaesthetics are widely used in biomedical research, yet increasing evidence shows that their effects on the brain and related systems extend beyond transient loss of consciousness. Although the impact of single anaesthetic exposures has been extensively examined, the biological consequences of repeated anaesthesia remain poorly defined.

Aims: This study aimed to determine how brief, repeated isoflurane exposures influence behaviour, cerebrovascular function, and microglial phenotype in adult mice, and to identify sex-dependent differences in these outcomes.

Methods: Adult male and female CD1 mice underwent a series of short isoflurane exposures replicating common laboratory protocols. Behaviour was assessed using open-field locomotion, nesting, and burrowing tests. Cerebral blood flow, endothelial markers and glial states were analysed to characterise vascular and immune responses.

Results: Repeated anaesthesia induced distinct behavioural, vascular, and cellular changes. Females showed reduced locomotor activity, whereas both sexes exhibited impaired nesting and burrowing performance. Cerebrovascular responses were affected by anaesthesia, but not directly by the repeated exposure. But repeated anaesthesia altered endothelial marker expression and shifted microglia toward a hyporeactive phenotype.

Discussion/Conclusions: These findings show that even short, routine anaesthetic exposures lead to meaningful alterations in neural, vascular, and immune systems. Anaesthetic regimen is therefore an important experimental variable that warrants careful consideration to improve reproducibility and interpretation in preclinical research.

WEB-BASED PLATFORMS ARE A VALUABLE TOOL FOR MANAGING PRECLINICAL ANIMAL STUDIES

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Web-based platforms offer features such as data capture, reporting, and collaboration, all of which are crucial to the success of preclinical research. These platforms are keen to reduce administration and fit compliance with regulatory standards.

The contemporary ecosystem of web-based systems for preclinical research can be divided into five categories: (1) GLP-grade preclinical study suites, (2) enterprise Electronic Laboratory Notebook (ELN)/Laboratory Information Management Systems (LIMS) with in-vivo modules, (3) non-GLP operational workflow managers, (4) academic preregistration registries, and (5) web-based analytical/visualization engines.

Across categories, EU on animal use (Directive 2010/63/EU) gives the 3Rs legal force. Digital systems can operationalize the 3Rs in concrete ways: preregistration reduces duplication (Reduction) and sharpens design details that promote humane, bias-minimized study conduct (Refinement); enterprise platforms with integrated vivarium links, dosing histories, and threshold alerts help codify humane endpoints and severity tracking (Refinement); and modules that facilitate justified use of historical controls can lower concurrent control animal counts where scientifically appropriate (Reduction). Software cannot deliver Replacement on its own. Modern digital platforms increasingly support Reduction by enhancing planning, data sharing, and analytical power—helping research become both more humane and more scientifically efficient.

Together, these categories are reshaping academic preclinical research by aligning digital practice with reproducibility, 3R obligations, and, where pursued, regulatory readiness.

Modern preclinical research has become increasingly data-intensive, regulation-heavy, and collaborative, making traditional spreadsheet-based or paper-based workflows insufficient. Web-based platforms now play a growing role in ensuring study integrity, reproducibility, and operational efficiency across animal research. As preclinical studies continue to grow in scale and complexity, these tools provide the backbone for efficient, ethical, and scientifically robust animal research.

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SUPPORTING THE 3RS BY INCREASING CATHETER PATENCY DURATION IN RODENTS

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Vascular access is a key element in many preclinical studies. In chronic protocols involving repeated administration of compounds, blood sampling, or both, maintaining a prolonged catheter patency duration is essential. Several factors influence the catheter functionality, the most critical being the accurate positioning of the catheter tip at an optimal anatomical site. The use of a closed system is another crucial determinant; closed systems incorporating transcutaneous buttons have been shown to significantly extend catheter patency performance. Catheter material and tip design also play important roles. Polyurethane is widely considered the material of choice, while a rounded tip minimizes endothelial damage and reduces the risk of thrombus formation. Strict adherence to aseptic technique and the use of sterile solutions are essential to prevent infection and biofilm formation, thereby promoting an unobstructed catheter flow. In addition, employing a positive pressure technique during catheter locking decreases the likelihood of intraluminal clot formation. Collectively, these factors must be carefully considered and consistently applied to achieve optimal catheter patency duration.

Beyond extending the openness period of the catheter, the transcutaneous button offers additional advantages that support the principles of the 3Rs. With respect to Refinement, the protective metal cap covering the button enables group housing, improving animal welfare and respecting rodents' natural social behaviors. The transcutaneous button also reduces animal handling and associated stress, contributing to enhanced well-being and better data quality. In terms of Reduction, prolonged catheter patency duration decreases the number of animals subjected to surgical procedures and enrolled in studies, thereby supporting more ethical and efficient chronic research designs.



EDUCATION and TRAINING FOR THE FUTURE: SKILLS, ETHICS & MENTORSHIP

YOU CAN'T CHOOSE WHAT YOU'VE NEVER SEEN: REINTRODUCING LABORATORY ANIMAL MEDICINE TO VETERINARY EDUCATION PROGRAMS

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Introduction: Laboratory animal medicine is underrepresented in veterinary education, as curricula are predominantly focused on companion and food animal practice. Consequently, veterinary students complete their education with little to no exposure to the field. This limited visibility contributes to persistent misconceptions and stigma surrounding animal research, including misunderstandings about animal care, regulatory oversight, and the veterinarian's role in safeguarding animal welfare. These factors contribute not only to declining recruitment into laboratory animal medicine but also to broader gaps in professional understanding among veterinary graduates.

Aims: To develop and evaluate a 4-week rotation in laboratory animal medicine designed to increase student exposure, address misconceptions about animal research through direct, mentored experience, and improve understanding of the ethical, regulatory, and clinical frameworks governing laboratory animal care.

Materials and Methods: A 4-week final-year clinical rotation in laboratory animal medicine was developed for students from our institution's college of veterinary medicine. The rotation fulfilled core professional activities while providing comprehensive exposure to the specialty. Learning objectives, adapted from the ACLAM role delineation document, included IACUC protocol review, clinical care, anesthesia, surgery, necropsy, and facility management. Implementation required institutional support, strategic scheduling, and iterative refinement based on student feedback. Targeted outreach emphasized hands-on experiences and exposure to diverse species.

Results: Over three years, enrollment increased by 78%. Most students reported no prior exposure to laboratory animal medicine. Post-rotation feedback demonstrated high satisfaction, improved understanding of the field, and strong appreciation for the emphasis on animal welfare and respectful animal care. Hands-on clinical engagement and mentorship were consistently identified as key drivers of positive perception.

Conclusion: Distributive clinical education models can be leveraged to expand exposure to laboratory animal medicine while addressing misconceptions and improving recruitment. Structured, competency-based rotations offer a scalable strategy to educate veterinary professionals and strengthen the workforce pipeline in modern veterinary curricula.



OPTIMIZING RESOURCES: DO WE REALLY NEED A DIRTY NURSE IN EXPERIMENTAL SURGICAL PROTOCOLS?

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One of the obligations of a researcher is the responsible conduct of research. To attain this, one must try to optimize resources, may they be financial, material and/or human. A frequent and divergent discussion in research facilities is the use of dirty nurses in research protocols. A dirty nurse is defined as a person dedicated to the dirty (not sterile) part of the realization of an experimental surgical protocol in which we find a strict sterile part. A dirty nurse will realize the anesthesia, preparation, positioning of an animal in a surgical protocol, not to forget the postoperative recovery. The sterile nurse or surgeon will only perform the sterile part of the protocol including material set-up, surgery and preparation of the next surgery (new material, suture, instruments, etc.).

Should we include dirty nurses in studies or is a single person efficient at realizing all steps of an experimental protocol? I believe that if we want to optimize resources, the use of a dirty nurse is imperative. Instead of performing 6 surgeries in one day, we can perform 24 to 30 when using a dirty nurse. I will illustrate with a real time video, the choreography of the tasks performed by a team of 2 in a research setting using the destabilization of the medial meniscus (DMM) model in mice.

In this fashion, the best of care is conducted by the dirty nurse, including recovery surveillance, The cross contamination is minimized, thus reducing the post-operative infections. The realization of surgeries in one day also refines the post-operative evaluations which can all be performed at the same timepoints, minimized the bias of time in experimental protocols.



REFINE TO REDUCE: ANAESTHESIA AND TECHNIQUE

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Safe, well-designed anaesthetic protocols represent one of the most effective refinement strategies capable of directly reducing animal numbers in preclinical research. Mortality during anesthesia in healthy laboratory rodents is generally expected to be exceptionally low and is typically reported to be well below 1% (1). Yet preventable losses still occur due to inadequate preparation, insufficient monitoring, and avoidable physiological disturbances. Implementing structured refinement—beginning with the PREPARE and ARRIVE frameworks—reduces periprocedural mortality, improves data quality, and ensures that no animal life is unnecessarily wasted.

Three domains are central to achieving Reduction: optimised anaesthetic protocols, comprehensive monitoring, and proactive prevention of vagal inhibition. Modern anesthesia requires balancing the anesthetic triad—hypnosis, analgesia, and muscle relaxation—while recognizing that different agents contribute unevenly to these components. In laboratory rodents, inhalational agents such as isoflurane provide rapid titration but limited analgesia; therefore, thoughtful premedication and robust perioperative pain management are essential. The appropriate use of sedatives, opioids, and local anaesthetics minimizes stress responses, stabilizes physiological parameters, and reduces experimental variability, thereby lowering the number of animals required to achieve reliable outcomes.

Monitoring forms the second pillar of refinement. Continuous assessment of reflexes, respiratory and cardiovascular function, body temperature, and depth of anaesthesia—supported by pulse oximetry, temperature probes, ECG, or respiratory pillows—enables early recognition of complications. Preventing hypothermia, hypoxia, and hypovolemia is fundamental to both animal welfare and scientific validity.

Finally, vagal inhibition is an under-recognised but critical cause of anaesthetic mortality. Common stimuli such as laryngeal manipulation, ocular pressure, visceral traction, or α_2 -agonist premedication may provoke severe bradycardia or asystole. Understanding these triggers, maintaining adequate anaesthetic depth, and having anticholinergic or reversal agents readily available are essential measures to prevent fatal events.

Refining anaesthesia, monitoring, and technique not only safeguards welfare but directly supports Reduction, ensuring that every animal contributes meaningful scientific value.

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CTRL+ALT+DELEGATE: LEVERAGING AI TOOLS TO BOOST NACWO, NIO, NTCO AND VETERINARIAN ROLES AT LABORATORY ANIMAL FACILITIES

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Introduction: Named Animal Care and Welfare Officers (NACWOs), Named Training and Competency Officers (NTCOs), Named Information Officers (NIOs) and Veterinarians are the cornerstone roles at each facility conducting animal research. These professionals ensure regulatory compliance, champion animal welfare and the 3Rs, overseeing staff training and competency and promoting a Culture of Care. Yet these vital professionals face mounting challenges: escalating administrative workloads, the demand for clear and engaging training content, and the complexity of maintaining effective communication across diverse stakeholder group which can all reduce the time dedicated to their core missions. Here we present concrete examples on how AI tools can help address these issues.

Material and Methods: At AstraZeneca, we embarked on an exploration of a set of different AI-powered tools to streamline operational demands and enhance the role of our named officers: automatically generate comprehensive compliance meeting minutes, accelerate the review and modernisation of regulatory training, and create diverse educational materials (including presentations, interactive quizzes, procedural checklists, and FAQ documents) directly from legislation, institutional policies and standard operating procedures. Additionally, these tools can synthesise complex animal welfare regulations and accreditation requirements into accessible summaries for the broader *in vivo* community. Building on these successes, we have expanded our AI toolkit to include further capabilities. AI-generated training videos provide information and training with engaging visual narratives, while specialized AI agents can assist in writing, review and improve regulatory documents (ex: Licences and permits, SOPs, Work instructions) consistent with national and European legislation and AAALAC accreditation requirements.

Conclusion: However, AI tools works best as a smart assistant, not a replacement for professional judgment. AI tools don't replace named officers but, when used thoughtfully with robust oversight, they free capacity for meaningful work requiring human compassion, expertise, and judgment, ultimately advancing animal welfare and the 3Rs.



IMPLEMENTING A HARMONIZED CONTINUING PROFESSIONAL DEVELOPMENT FRAMEWORK TO ENHANCE COMPETENCE OF ANIMAL RESEARCH PROFESSIONALS

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A structured continuing professional development (CPD) process for professionals involved in animal research is essential to ensure sustained competence, high standards of practice, and ongoing improvements in animal welfare and laboratory animal science. FELASA – Federation of European Laboratory Animals Sciences Associations – has published recommendations for a lean, transparent, and harmonised framework designed to improve CPD across all functions, roles, and responsibilities defined under Directive 2010/63/EU. Continuing professional development is conceived as a career-long process that begins after completion of basic training and achievement of initial competence.

Central to the framework is the creation of an individual continuing professional development portfolio, aligned with a personal development plan and regularly reviewed by the person responsible for training and competency. The portfolio provides a structured method for documenting learning activities, reflecting on professional growth, and assessing maintained competence. Institutions are encouraged to integrate this system into routine monitoring processes, with formal competency reviews recommended at intervals not exceeding 5 years.

The recommendations further address the scope and diversity of eligible development activities and discuss expectations regarding the minimum level of engagement required to demonstrate ongoing professional growth. To promote consistency and mutual recognition, essential information that should accompany certificates of participation is defined, enabling transparent evaluation of continuing professional development across organisations and countries.

Overall, implementation of a harmonised strategy for planning, recording, and reviewing continuing professional development supports professional mobility, strengthens institutional quality assurance, motivates staff, and fosters a strong culture of care, ultimately contributing to responsible, ethical, and high-quality animal research practices.



EMERGING JOINT REGIONAL 3RS CENTER IN SPLIT, CROATIA – COST ACTION (CA) IMPROVE INITIATIVE

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The CA IMPROVE initiative's goal in the last year of its scientific mandate (2026) is to establish a joint regional 3R center at the Medical Faculty Split (MEFST), University of Split, Croatia. The 3Rs centres and platforms are very important points of contact and play an important role in their respective countries as 'on the ground' facilitators of Directive 2010/63/EU. The joint 3R center aims to serve the Balkan region and its non-EU and EU member countries under the umbrella of European network of 3R Centers (EU3Rnet) by provision of continual professional development (CPD) opportunities in biomedical and Laboratory Animal Science (LAS) field. With its state-of-the-art laboratory animal facility and congress center, MEFST can facilitate international collaboration and act as a regional networking hub.

The joint 3R center aims to accelerate the implementation of the 3Rs and animal-free new approach methodologies (replacements) through expert advice and provision of 2010/63/EU aligned education and training tailored to the specific research and training needs and aims of each country or region, enabling conduct of high-quality scientific research throughout Europe. Furthermore, it aims to provide its members with expert consultations to help them steer through *in vivo* Project Applications' and Authorisations' requirements and *in vivo* project ethical evaluations. The center aims to host international and diverse stakeholders from academia, industry, legislative bodies, biomedical associations and like and they all stand to benefit from the center's short-term and long-term goals and deliverables compliant with 2010/63/EU requirements and best scientific and (laboratory animal) veterinary research practices.

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SMARTER SUBMISSIONS: AI-POWERED PRE-ETHICS REVIEW TO ENHANCE PROTOCOL QUALITY

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Introduction: Ethical review of animal research protocols is essential, yet committees often receive submissions with inconsistent quality, missing methodological details, and variable adherence to the Israeli Animal Welfare Law and the *Guide for the Care and Use of Laboratory Animals*. These gaps create delays and repeated revision cycles. AI tools offer an opportunity to improve early-stage review. Etiq-Tech aims to pair deterministic data validation with grounded AI guidance so that protocols arrive aligned with regulatory and refinement expectations while preserving committee authority.

Aims: To develop an AI-based co-reviewer that evaluates animal-use ethics applications, identifies omissions and non-compliance, and returns immediate, structured, evidence-based corrections linked to the relevant regulation or refinement principle, improving protocol quality before committee submission.

Materials and Methods: Etiq-Tech uses a layered design. A parser converts RTL-formatted protocol exports into a unified structured object for programmatic checking. A deterministic linter and renderer apply word limits, reconcile animal numbers, enforce monitoring according to severity, verify analgesia and housing details, activate relevant sections when needed, and generate compliant output. A metadata catalog stores exemplars and prompt templates that integrate schema rules, linter findings, and grounded text. An evidence layer indexes refinement literature, Israeli regulatory clauses, and institutional guidance to provide traceable citations. Batch and regression tools maintain consistency across updates.

Results: The system reliably detects missing information and non-compliance, improving clarity and completeness before committee review. Users report better understanding of expectations and faster revision. Parsing, issue detection, rendering, and automated prompting function consistently. The evidence engine provides structured corrections with governing citations and confidence metadata.

Conclusion: An AI-based co-reviewer can strengthen the quality, compliance, and refinement of animal research protocols, supporting ethical integrity and committee efficiency.

ETHICS, 3R PRINCIPLES AND SIMULATION-BASED LEARNING FOR ZONOTIC PREPAREDNESS IN VETERINARY EDUCATION

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Introduction: Veterinary education requires ethical decision-making, animal-welfare responsibility and preparedness for managing zoonotic risks, while training systems are increasingly expected to align with the 3R principles (replacement, reduction, refinement). This study presents an integrated curriculum model in which ethics, the 3Rs and One Health reasoning function as measurable competencies, avoiding the routine use of laboratory animals.

Aim: To develop an integrated, simulation-based and research-oriented education model that strengthens zoonotic preparedness, biosafety behaviour and analytical reasoning, while embedding the 3Rs as operational professional competencies across an 11-semester veterinary curriculum.

Materials and Methods: The model is implemented within the Integrated Master of Veterinary Medicine programme (5.5 years; 347 ECTS) and emphasises a One Health approach using a backward-design framework. Ethics-, 3R- and zoonosis-related outcomes guide the selection of learning activities and assessment tools. Simulation-based training utilises task trainers, synthetic models, virtual cases and standardised scenarios addressing specimen collection, containment, PPE use, infection control, triage and risk communication, and is assessed through OSCEs and structured checklists. Research-oriented learning is based on slaughterhouse-derived diagnostic materials, archival resources, authentic surveillance datasets and data-driven analysis in biostatistics, bioinformatics and GIS. Workflows from the SRNSFG project FR-22-6780 are integrated as authentic learning resources. Portfolios and longitudinal monitoring support progressive competency development.

Results: The model generates (i) an ethics- and 3R-competency map; (ii) a portfolio of simulation activities with measurable OSCE outcomes; and (iii) a non-animal research learning trajectory linking fieldwork, diagnostic reasoning and computational analysis. Engagement with surveillance workflows improves biosafety awareness, analytical judgement and zoonotic risk communication.

Conclusion: The model enhances zoonotic preparedness and evidence-based competence while avoiding routine laboratory-animal use, offering a transferable approach that supports animal welfare, educational quality and public trust.

Acknowledgements: *The work was supported by Shota Rustaveli National Scientific-Research Foundation of Georgia (SRNSFG) [Grant Number FR-22-6780]*

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FROM COMMUNITY TO COMMUNITY: STRENGTHENING KNOWLEDGE AND COMPETENCE ACROSS EUROPE

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The Education and Training Platform for Laboratory Animal Science (ETPLAS) aims to strengthen excellence and harmonisation in laboratory animal science across Europe by connecting people, improving knowledge and promoting competence. Through the development and sharing of high-quality education and training resources, collaborative networks, and best practices, ETPLAS supports professionals, institutions and regulators in advancing consistent standards and promoting continuous professional development (CPD). By fostering cooperation between stakeholders and facilitating access to innovative learning opportunities, the platform contributes to improved skills, ethical practice and scientific quality.

ETPLAS offers training solutions for authorities, accreditors, establishments, course organisers and individuals. E-learning modules, that can be used for blended learning, competence assessment tools, a course directory of registered LAS courses, a secure online examination platform are available. These resources facilitate consistent standards, mutual recognition, and trustworthy competence across Member States, while supporting CPD requirements and lifelong learning. Through shared materials and standardised assessments, ETPLAS helps ensure robust, reliable and continually improving training outcomes.

This presentation will outline ETPLAS's services, impact and future priorities, and highlight opportunities for community engagement in shaping the next generation of LAS education and training across Europe.



3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

SEX DIFFERENCES IN THE PHARMACOKINETICS OF BUPIVACAINE SHOULD BE CONSIDERED WHEN TREATING PIGS

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Background: Bupivacaine is a widely used local anaesthetic, clinical and preclinical studies have suggested potential sex-related differences in its pharmacokinetics, particularly in absorption and elimination phases, though the findings remain inconsistent. In rodent models, females often display lower peak plasma concentrations and slower elimination compared to males¹. However, limited data exist in pigs, a key translational model for evaluating new local anaesthetic formulations. This study aimed to assess sex-related differences in bupivacaine pharmacokinetics and local tissue characteristics following wound infiltration in Göttingen minipigs.

Methods: Male and female Göttingen minipigs were anesthetized, and a standardized 7 cm flank full skin incision was made^{2,3}. Bupivacaine (8 mg/kg) was administered into the wound space. Blood samples were collected at defined time points for quantification of plasma bupivacaine levels. Skin samples from the injection site were processed for histological examination (H&E and IHC staining) to measure epidermal, dermal, and subcutaneous fat layer thickness as well as blood vessels density.

Results: Plasma bupivacaine concentrations were significantly higher in females compared with males (6480 ng/mL vs. 4600 ng/mL; $p < 0.001$). The time to reach peak concentration was 30 minutes in females and 15 minutes in males, indicating slower absorption in females. Histological analysis revealed no sex-related differences in epidermal or dermal thickness; however, females exhibited a thicker subcutaneous fat layer ($2929 \pm 167 \mu\text{m}$ vs. $2326 \pm 1394 \mu\text{m}$; $p < 0.01$). No correlation was observed between body weight and fat layer thickness. Ongoing analyses are evaluating potential sex differences in local vascular density to further elucidate absorption kinetics.

Conclusion: Female minipigs exhibited higher systemic exposure and delayed peak levels following local bupivacaine administration which may put them in higher risk for cardiovascular toxicity. Differences in tissue composition may underlie the observed pharmacokinetic sex related difference.

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SMALL STEPS, BIG IMPACT: IMPROVING ASEPSIS TIPS FOR RODENT SURGICAL PROCEDURES

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The principle of aseptic surgery, established at the end of the 19th century, allows to prevent contamination with microorganisms during a surgery and associated post-operative complications.

This fundamental principle applies to human and veterinary medicine. In the context of research projects, strict aseptic conditions do not only allow preventing healing issues of operated individuals but also avoid bias in collected scientific data.

Strict aseptic conditions are usually respected for large laboratory animals' surgeries. However, it remains significantly improvable when it comes to rodents. Rodent surgeries carry their own challenges and some of the large animals' aseptic techniques are not easy to implement in rodents. But specific strategies can be established, without impairing surgery in series cost and efficiency.

During this presentation, pragmatic and affordable tips regarding prevention of contamination from potential sources (environment, animals, instruments, and surgeons) will be presented.

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FELASA WG ON BENCHMARKING TECHNICAL STAFF RATIOS FOR LABORATORY MOUSE CARE: PRELIMINARY FINDINGS

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Laboratory mice husbandry and care primarily rely on technical personnel. Across facilities, differences in housing conditions, husbandry practices, and technological advancements require varied operations management and staffing levels. The FELASA Working Group conducts a benchmarking exercise to understand staffing practices according to personnel's role and husbandry conditions.

We developed two surveys to collect data on staffing numbers and task-based time allocation. The first survey explored general management practices, while the second focused on task-based workload. Initial exploratory findings revealed substantial variation in facility operations and perceptions of optimal staffing. Absenteeism and increased workload were identified critical challenges; these issues significantly affect continuity of care and operational stability. Preliminary data indicate that 65% of respondents perceived a workload increase, while 72% identified absenteeism as a major issue.

The follow-up survey was designed to investigate specific operational requirements for animal caretakers, animal technicians and animal technologists¹. Its purpose is to capture workload associated with animal care and supporting activities, categorized by task types and various technical staff roles.

We will present the preliminary results from both surveys. Our analysis aims to provide evidence-based guidance for appropriate technical staff workforce design for laboratory mouse housing and care. These findings may support facilities assess staffing levels, identify workforce gaps, implement corrective actions, and harmonized staffing practices in mouse laboratory animal facilities, ultimately enhancing animal welfare and scientific quality.

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NAMS IN ACTION: REDUCING ANIMAL TESTING THROUGH INTEGRATED IN SILICO STRATEGIES

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The integration of in silico toxicology into early-stage drug development marks a paradigm shift toward more ethical, efficient, and predictive research. This study explores the strategic application of computational methods – including quantitative structure-activity relationship (QSAR) modeling and activity prediction algorithms (PASS), along with pharmacokinetic and pharmacodynamic simulations (SwissADME, GUSAR) – for evaluating novel active pharmaceutical ingredients (APIs). Leveraging these tools during lead optimization allows effective prioritization of compounds with favorable safety profiles and eliminates high-risk candidates prior to any in vivo experiment. Our case studies demonstrate that this systematic implementation reduces animal use in preclinical safety testing by 40–70%, directly advancing the 3Rs principles. We describe frameworks that address model transparency and applicability to ensure regulatory acceptance, highlighting integration pathways where computational predictions complement targeted in vitro assays. The methodology has been successfully applied during the development of a series of pharmaceutical products for external use, which are now progressing to clinical studies.

This successful translation from in silico prediction to clinical candidates underscores a broader transition in toxicology, which is being supported by an evolving regulatory landscape that increasingly endorses validated computational approaches. Ultimately, the purposeful adoption of predictive toxicology establishes computational methods as critical gatekeepers before animal studies begin. This not only accelerates development timelines and lowers costs but also defines the future of ethical drug development through the harmonious integration of computational methods and responsible use of laboratory animals.



REFINING IN ACTION: A COMPARISON OF HYPOBARIC AND NORMOBARIC EXPERIMENTAL HYPOXIA MODELS IN PERINATAL RATS

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Reducing Animal Use: Sharing Resources, Avoiding “Wasted Life”

A variety of animal models have been developed to study perinatal hypoxic–ischemic (HI) brain injury and evaluate therapeutic interventions. The Rice–Vannucci model, involving unilateral carotid ligation in P7 rats, has been the gold standard for decades but is invasive and non-physiological. Hypoxia-only models provide ethically refined alternatives, allowing study of milder, clinically relevant injury while reducing animal distress.

This study aims to compare hypobaric hypoxia (HH) and normobaric hypoxia (NH) induced on postnatal day 1 (P1) in rats, and to compare their translational relevance for modeling mild perinatal hypoxic events.

In HH, 52 Wistar Han pups (RccHan: WIST) (3 females and 3 males per experiment) were exposed for 2 hours to hypobaric (pO₂ 9.7kPa; pATM 46.7kPa) or control (pO₂ 21.2kPa; pATM 101.3kPa) conditions¹. In NH, 76 pups (4 females and 4 males per experiment) were exposed for 2 hours to hypoxic (8% O₂ in 92% N₂) or control (21% O₂) conditions, with continuous monitoring of oxygen, temperature, pressure, and humidity². Following exposure, pups were assessed for acute metabolism or returned to dams for subsequent behavioral testing of exploration, sociability, and learning during adolescence (P33–P43).

Acute metabolic analysis confirmed hypoxic stress in both models, with reduced base excess and bicarbonate, increased blood lactate, and preserved systemic pH. Perinatal hypoxia consistently increased exploratory rearing. HH induced broader, sex-specific effects, including increased locomotion, reduced sociability in females, and transient learning impairment in males. NH produced subtler but reproducible behavioral changes without impairing learning.

NH was identified as the mildest perinatal intervention capable of inducing measurable neurobehavioral alterations. By avoiding invasive ischemic procedures while maintaining translational relevance, NH represents an ethically refined, scientifically robust model. This approach exemplifies practical 3Rs implementation, particularly Refinement and Reduction, maximizing the scientific value of each animal used.

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SEX AS A BIOLOGICAL VARIABLE IN LABORATORY RESEARCH: OBSERVATIONS FROM COLLABORATIVE RESEARCH ENVIRONMENTS

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The failure to consider sex as a biological variable (SABV) in preclinical and clinical research has led to clinically relevant consequences, particularly for women. Biological sex differences influencing pharmacokinetics and pharmacodynamics have resulted in women being systematically exposed to higher effective drug doses, increasing the risk of chronic overmedication and adverse drug reactions (ADRs)¹. Reports of certain drugs withdrawn by the U.S. Food and Drug Administration (FDA) due to safety concerns suggest they may pose a greater risk in women, highlighting potential issues with testing therapies primarily in male subjects.

Several arguments have historically been used to justify sex bias in biomedical research. In preclinical studies, the estrous cycle in females has often been cited as a reason to exclude them from studies. In contrast, in some cases, researchers have avoided using male animals due to concerns about aggression. In clinical research, the protection of women of reproductive age has long been used to justify excluding them from early-phase trials, further reinforcing sex bias in evidence generation. Such practices have sometimes persisted due to methodological inertia or misinterpretation of the 3Rs principles^{2,3}.

Recognition of this problem has led to policy changes, including the National Institutes of Health (NIH) requirement (NOT-OD-15-102) to address SABV in grant applications. These changes are increasingly visible at the local level, reflected in sex-inclusive preclinical and translational studies conducted within our Institute and collaborating institutions, demonstrating that implementation of SABV is both feasible and scientifically informative.

Despite these advances, sex bias remains prevalent. Addressing this gap requires continuous education, interdisciplinary dialogue, and changes in everyday research practice. Initiatives such as COST Action CA24168 – *European Initiative to Enhance the Current SABV Policy in Preclinical Biomedical Research (EU-SABV)* support these efforts and promote the integration of SABV into laboratory animal research.

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ONE WELFARE

CULTURE OF CARE: REALISTIC IMPLEMENTATION IN THE EUROPEAN FRAMEWORK

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Implementing a culture of care in research animal facilities has become essential over the past decades – not only to safeguard animal welfare, but also to uphold the integrity of scientific research and ensure the wellbeing of all personnel involved.

A culture of care is defined as a shared commitment to the ethical treatment of animals, encompassing attitudes, behaviors, and practices that prioritize animal welfare and responsible research.

This presentation will provide a comprehensive overview of practical and effective strategies for fostering a culture of care within European research animal facilities, illustrated through real-world examples drawn from recent AAALAC International site visits across Europe.

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

Key elements such as staff training, ethical review processes, continuous improvement, and the practical application of the 3Rs will be discussed. The critical role of Animal Welfare Bodies (AWBs) and Ethics Committees (ECs) in promoting and monitoring a culture of care will be highlighted, emphasizing their importance in ensuring compliance and driving ongoing progress.

Additionally, we will address the importance of teamwork and the active involvement of all stakeholders—from researchers to animal care staff—in creating a supportive and collaborative environment.

Attendees will gain valuable insights into best practices and innovative approaches that have been successfully implemented across diverse institutions.

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WHAT HAVE SEA-KAYAKING AND WORKING IN ANIMAL RESEARCH IN COMMON?

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At first glance, sea-kayaking and animal research appear to have little in common. However, both depend on effective leadership, clear communication and shared responsibility to ensure safety, welfare and successful outcomes. This presentation uses a sea-kayaking journey as a metaphor to explore how leadership and communication practices from this demanding activity can be translated into the culture of animal research.

Leadership and communication are two-way processes. An effective sea-kayak leader must encourage individuals to develop their skills through appropriate challenge while preventing fear and maintaining safety. Support is provided when needed, but individuals are also given the opportunity to achieve independently. These principles closely mirror good leadership practice within animal research environments.

Teamwork is critical. Leaders must understand the skills, limitations and personalities of all group members and know how best to use these in an emergency. Imagine a howling wind and a choppy sea, with two kayakers capsized near rocks. Everyone must understand their role in keeping those in the water, and the wider group, safe—whether through rapid recovery, stabilisation or recognising when external rescue is required. Training prepares teams for such scenarios, but clear and timely communication is equally essential.

Individuals also carry responsibility for accurately assessing their own abilities. Overestimating competence can place the entire group at risk. Respect for the skills and experience of others is vital, and all must feel empowered to raise concerns. This presentation translates these lessons into the animal research environment, highlighting their relevance to leadership strategies for ethical culture change, culture of care, training, welfare, teamwork, mutual respect and the consequences of getting it wrong, and recognising that animal welfare and human well-being are deeply interconnected.



EXAMINATION OF MENTAL WELL-BEING AMONG LABORATORY ANIMAL RESEARCH WORKERS

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Introduction: In addition to the ethical and professional concerns surrounding animal experimentation, increasing attention is being directed toward the psychological burdens experienced by professionals directly involved in such research. Personnel working in animal facilities—technicians, caretakers, researchers, and veterinarians—interact closely with experimental animals daily; in the case of prolonged studies, personal attachments may even develop, and they are often involved in the animals' euthanasia. This duality—the provision of care for the animals contrasted with their sacrifice for scientific purposes—is termed the "caring-killing paradox" in the literature and constitutes a primary source of compassion fatigue (CF), secondary traumatization, and moral distress.

The concept of compassion fatigue was originally described in helping professions (e.g., healthcare and social work), where prolonged exposure to clients' suffering can lead to heightened psychological exhaustion. Over the past decade, however, a growing body of studies has demonstrated that CF is also highly prevalent among laboratory animal research staff.

Materials and Methods: The objective of our study is to map the construct of compassion fatigue among Hungarian laboratory animal research professionals and to assess the psychological burden on these workers. The questionnaire items were grouped into seven dimensions: Moral Distress, Ethical Conflict, Euthanasia Distress, Animal Suffering Disposal, Compassion Fatigue, Role Conflict, and Compassion Satisfaction, with six questions per dimension. To refine the questionnaire and enhance its validity, five experts evaluated the compiled material based on conceptual clarity and comprehensibility: two psychologists, two mid-career professionals working with laboratory animals, and one assistant with 15 years of experience in animal care, research, and teaching. The revised questionnaire will be distributed to as many institutions as possible to capture the phenomenon of compassion fatigue across all levels of laboratory animal staff. At the conference, we aim to present both the questionnaire and preliminary findings.

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HANDLING METHOD OF LABORATORY MICE HAS EFFECT ON THEIR EXPLORATORY BEHAVIOUR

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Routine work with laboratory animals involves handling that cause discomfort and stress. The tail picking is the method most commonly used with mice. Response of the mouse to the tail picking is a diminished interest in exploring their surroundings. This can have a significant impact on the results of behaviour research and the behaviour of animals in various experiments, but also on the results of research. The introduction of non-aversive method of handling, tunnel handling, can help to reduce differences and deviations. During the study, we investigate how the constant presence of a tunnel in a cage affects behaviour and how long it takes for mice to get used to handling. In the study we use three different mouse lines: FHI, FLI and BALB/c and each mouse line consisted of six animals, including three males and three females. Each line was handled in all three different ways (tail, home tunnel, foreign tunnel). Before, during, and after handling, we performed an open field test and an elevated plus maze test with all animals. From the results, it appears that mice handled by the tail during the open field test spent more time immobile in the centre of the arena, whereas mice handled using a tunnel were much more active and explored the periphery more extensively; they also showed reduced urination and defecation. In the elevated plus maze test, we observed immobility and reduced exploration of the open arms in mice handled by tail picking. No differences were detected between mice that had continuous access to a tunnel in the cage and those that were exposed to the tunnel only during handling. The results indicate that tunnel handling reduces anxiety-like behaviour and promotes exploratory activity compared to tail picking, while only minor differences related to sex and mouse line were observed.



CHALLENGES IN USING AGED ANIMAL MODELS FOR NEURODEGENERATIVE DISEASE RESEARCH

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Introduction: Neurodegenerative diseases are challenging for modelling – they rarely occur spontaneously in animals. Advanced age is the most significant risk factor for conditions such as Parkinson's and Alzheimer's disease, the use of aged animals in preclinical research remains limited due to experimental, time, and cost-related challenges. Cats and dogs develop a similar condition to Alzheimer's disease, yet drug testing is limited to clinical patients for their own benefit rather than for advancing general scientific knowledge. Few studies have demonstrated age-related beta amyloid plaques and neuronal loss in rodents; however, researchers rely on induced models that cannot fully recapitulate the slow progression and complex pathology observed in humans.

Aims: Find best rodent model for preclinical testing of butyrylcholinesterase inhibitors after in vitro modelling.

Material and Methods: Ageing C57BL/6 and Balb/c mice of both sexes were compared to chemically induced cognitive impaired mice. Male 15–16-month old C57BL/6 mice were given the effective dose orally and tested in elevated plus maze, novel object recognition and modified Barnes maze. Surplus mice were killed between 18 and 24 months of age and whole brain collected.

Results: The drug crossed the blood-brain barrier but failed to induce significant improvement in behavioural performance. Untreated mice showed a decrease in brain cortex thickness, hippocampal area, and an increase in amyloid plaques and nNOS-positive neurons were observed², raising questions about the choice of using younger mice for drug testing.

Conclusion: Studies performed on mice aged 2 years or older are extremely rare. According to a large animal supplier, 24-month old mice correspond approximately to 70-year old humans, with senescent changes in almost all biomarkers in all animals. In our case, the study design simply failed to account for the prolonged developmental timeline required to observe the full spectrum of neurodegenerative pathology. Later on testing (re)turned to induced and transgenic mice models³.

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ARE FRAUDULENT STUDIES IN PRECLINICAL MEDICINE LEADING US ASTRAY?

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There is a growing awareness of fabricated studies being passed off as real ones in scientific journals. The practice is not new, but with the ever-increasing pressures to publish, combined with a greater availability of computer software capable of aiding fraudsters (most recently, the explosive adoption of generative artificial intelligence - GenAI), the numbers of faked publications are reaching new highs. How will this affect our ability to make sense of scientific literature in our field going forward? And to which degree is laboratory animal science/preclinical medicine affected?

In the course of carrying out systematic reviews of rat studies in depression and pain, we have had the misfortune to uncover a great number of problematic – potentially fraudulent – studies. Having sifted through hundreds of papers across two unrelated fields of laboratory animal studies, we have found that 10-20 % of studies are unreliable; possibly even fabricated. These are shocking numbers to us – numbers that cannot be ignored.

In this talk, I will present what we found in our investigations. I will show what a problematic study looks like and explain why we sometimes suspect foul play. We have also established how a systematic review can be affected by the inclusion of problematic studies. I can offer no simple solutions to the problem, rather, my goal is to raise awareness of these issues, and to demonstrate that they are hiding in plain sight. We may all have read a peer-reviewed publication recently describing a study that never actually took place. Most likely we trusted that study. This begs the question: How do we make sure that our own research is not led astray by mistakenly believing in fraudulent studies?



DESIGNING FOR WELFARE: FACILITIES, TECHNOLOGY & SUSTAINABILITY

REFINING HUMANE ENDPOINT IDENTIFICATION THROUGH TIME-SERIES FORECASTING

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Introduction: Improving the severity assessment in animal research is crucial for refining animal research in line with the 3R (Reduce, Replace, Refine) principle. Objective and model-specific parameters, in combination with robust statistical and algorithmic procedures, are essential for enhancing the evaluation of animal distress.

Aim: Therefore, we want to expand the application of the RELative Severity Assessment (RELSA) procedure with our AutoRegressive Integrated Moving Average (ARIMA)-based foRcast tool. This tool is designed to identify individual animals at risk of humane endpoint thresholds by predicting future RELSA scores from previous observations.

Materials and Methods: To validate and evaluate the performance of this function, we re-analyzed the published data of, in total, 14 male and female C57BL/6 mice over five distinct animal models and interventions, including a sepsis model, two dextran sodium sulfate (DSS)-induced colitis models with different stressors, a pancreatic cancer model, and a neurosurgical intervention. The RELSA scores were calculated for each model with different outcome measures. These measures comprise transmitter-generated variables, such as heart rate, and behavioral parameters, such as voluntary wheel running.

Results: Humane endpoint RELSA scores ranged between 0.63 and 1.03, with the foRcast predictions achieving an overall root mean square error (RMSE) of 0.08 and a prediction interval coverage probability (PICP) of 91.67 %.

Conclusion: The results indicate an overall precise prediction of the RELSA scores at the humane endpoint and the general direction of the animal's development. At the same time, the foRcast function shows robust predictive behavior across the different animal models. Nevertheless, the results also highlight the intrinsic limitation of ARIMA models, that drastic changes are not reliably predictable. We assume higher-frequency measurements to reduce the impact of this limitation and achieve better predictions through incremental changes. Overall, the foRcast function demonstrates robust, precise predictive ability and is a valuable addition to 3Rs.

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PRECLINICAL IMAGING AT THE IPHYS BIOIMAGING FACILITY: THE CZECH-BIOIMAGING AND EURO-BIOIMAGING NODE MEMBER

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The IPHYS Bioimaging Facility is a strong partner in preclinical imaging, providing comprehensive support ranging from assistance with ethical protocol preparation and approval to access to advanced imaging techniques and methodologies. In collaboration with the Czech-BioImaging infrastructure, the facility offers a broad range of state-of-the-art technologies, including miniscopes, *in vivo* optical imaging and optogenetics (with 2P and 3P options), PET-CT modalities, and advanced MRI systems.

Research projects supported by the facility cover a wide spectrum, from behavioural studies using miniscopes, imaging of acute brain sections, and optical imaging of the brain through cranial windows, to metabolic studies of bone marrow using PET-CT scanning. In addition to research support, the facility organizes an annual course in preclinical imaging for researchers seeking to expand their knowledge of *in vivo* animal imaging.

Facility activities will be demonstrated through several case studies and user projects, illustrating how the IPHYS BioImaging Facility helps researchers enhance project effectiveness and efficiency while improving animal welfare. By enabling non-invasive, longitudinal imaging, the facility actively supports the 3Rs (particularly Refinement and Reduction): procedures are gentler and better monitored, the number of animals required can be reduced, and welfare is safeguarded through earlier detection of issues and more precise, data-driven interventions.



THE IMOUSE SYSTEM – VISUAL INSPECTION MEETS AI-BASED LONGITUDINAL ANALYSIS OF STANDARDIZED BEHAVIOURS

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In translational research, experimental animals remain standard for assessing the efficacy and safety of potential therapeutics. However, comprehensive longitudinal datasets are often not acquired, as manual data collection must be minimized to comply with the 3R principles. Moreover, visual observations and behavioural testing can disrupt resting periods, elevate stress levels, and introduce artificial outcomes.

We aim to tackle these unmet needs by implementing of the camera based digital monitoring system (iMouse) for animal husbandry and experimental use cases by upgrading existing laboratory equipment (retrofit). Here, we examine the impact of manual handling towards mice with no visible phenotype. Therefore, we recorded 6w of manual handling. We used 2w pre-experimental video material from 3 perspectives to train AI algorithms, followed by a time series analysis of standard and unusual behaviours during the 4w experimental data set.

In result, we showed that recorded video material in the pre-experimental phase was capable to train our existing models to detect behaviours for the specific strain with over 90% precision. Furthermore, we identified that unusual phenotypic behaviour during the experimental phase was induced directly by manual handling.

In summary, our results demonstrate that digital visual inspections of experimental animals are capable to identify a visible phenotype which has been overlooked by human based manual inspection for years. Thus, our findings lead to a better understanding of mouse models, reducing the bias through human handling. At the same time, we increase the data density and provide contact-free continuity of video surveillance.

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FIXING WHAT SLOWS US DOWN: SIMPLE DIGITAL SOLUTIONS FOR SMARTER ANIMAL CARE PROGRAMS

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Introduction: Oversight of laboratory animal facilities generates large volumes of operational and clinical data; however, these data are often fragmented across paper records, spreadsheets, or isolated software platforms. This fragmentation limits timely communication, hinders longitudinal tracking, and reduces the ability to make data-driven decisions that support animal welfare and program efficiency.

Aims: To implement and evaluate cost-effective, cloud-based digital solutions designed to improve data consolidation, communication, and veterinary care oversight in a laboratory animal program.

Materials and Methods: We developed and implemented low-cost digital tools using widely available cloud services, QR codes, and online survey formats. QR codes were deployed at the point of care to enable rapid access to standardized digital forms for veterinary notifications, husbandry operations, recharge tracking, pathology reporting, and procedural documentation. Submitted data were automatically consolidated into centralized cloud-based repositories, generating time-stamped records and searchable archives. These systems enabled real-time notifications to veterinary and research personnel and supported longitudinal tracking of clinical events, service utilization, and operational metrics.

Results: Implementation of these tools resulted in marked improvements in data accessibility, completeness, and timeliness. Veterinary notifications and researcher communications became standardized and traceable, reducing delays and information loss. Centralized data capture enabled reliable tracking of service recharges, pathology incidence, recurrence, and trends. Searchable archives facilitated retrospective analyses and supported data-driven adjustments to veterinary care, staffing, and resource allocation. All solutions were implemented at minimal to no financial cost and required no specialized hardware or proprietary software.

Conclusion: Low-cost, cloud-based digital innovations can substantially refine veterinary care delivery and program oversight by improving data consolidation, communication, and decision-making. By leveraging simple technologies such as QR codes and online forms, laboratory animal programs can enhance efficiency, transparency, and animal welfare without significant financial investment. These approaches represent a scalable refinement supporting proactive, data-driven veterinary care and continuous program improvement.

FROM PROCEDURE TO PRODUCTION: CREATING THE BIOEMTECH VEIN ACCESS INJECTION PLATFORM

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BIOEMTECH's "Vein Access Injection Platform" is an example of how a procedure implemented in the daily operation of a preclinical laboratory can lead to the creation of a novel commercial product. The setup for intravenous injections to the lateral tail vein of rats and mice includes various materials and equipment: intravenous catheters, syringes, gauze, heatpad, warm water and either a restrainer or anesthesia mask. Administration of radioactive materials requires additional provisions for radioprotection such as a lead syringe case. Daily administrations became an inspiration for creating a platform which accommodates all needs into a handy device. The use of a tailor-made purpose-specific injection platform promotes 3 important goals: (1) operator comfort and safety, (2) uniform setup and standardized procedure steps which ensure successful vein access and repeatability with significantly fewer deviations for more robust results, and (3) a refined approach for animal welfare.

Having a manufacturing department within the company makes the realization of the idea easier. Manufacturing and producing a device for preclinical research involves several risks, such as the cost and effort of production versus the market price, the small target customer pool and limited laboratory budgeting for certain products. Communication between departments, marketing and corporal support can lead to the launch of a new product despite the challenges. Such devices, designed by users for users, promote refinement and reproducibility of animal procedures and ultimately fill in a significant market requirement.



RELOCATION WITHOUT DISRUPTION: DO'S, DON'TS AND LESSONS LEARNED

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The Netherlands Institute for Neuroscience (NIN) recently completed a major renovation of its rodent facilities, comprising a mouse breeding unit and a rodent experimental unit, including a biosafety level 2 (BSL-2) area.

The NIN does not own the premises in which it operates, and the maximum capacity of the facility is ca 2100 cages (mice and rats). Modernization of rooms and equipment was required, but the key challenge was to ensure continuity of research activities throughout the renovation process.

The renovation was conducted in three phases. First, the breeding unit was closed and essential breeding activities were temporarily relocated to the old experimental unit to maintain experimental continuity. This phase was also used as an opportunity to improve genetic quality and health status of the mouse strains through rederivation or re-establishment via commercial vendors. Second, following renovation and disinfection through an external company, the new animal facility - comprising both breeding and experimental units - was commissioned, after which experimental equipment was cleaned, disinfected and moved. Finally, the old facility was closed, disinfected and returned to the property owner.

The entire process, from closure of the initial unit to full transition into the new facility and decommissioning of the old experimental unit, was completed within eight months.

Throughout this period, multiple operational and organizational aspects required careful management, including decisions on outsourcing services, allocation of personnel, negotiation of new researchers' requests for experimental space and time to continue their research, coordination with construction companies on technical requirements, management of the psychological impact of the relocation on PhD students and their research projects, and resolution of damages, errors, and unforeseen issues during the move.

This presentation will provide a stepwise overview of the process, highlighting key lessons learned and practical recommendations that may support other research institutions facing similar renovations challenges.



THE IMPACT OF PRE-EXPERIMENTAL HOUSING CONDITIONS ON SONG PRODUCTION IN CANARIES

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Introduction: Canaries are a powerful model for studying motor sequence learning due to their complex and flexible song repertoires. Successful neurophysiological recordings in singing birds require that birds resume singing shortly after electrode implantation and reliably produce high daily song output (~100 songs). Prior to experimentation, canaries are housed either in aviaries, which provide environmental enrichment and flight opportunities, or in smaller cages. A potential concern is that transferring birds from a large aviary to a confined acoustic recording chamber may disrupt song behavior more than transfer from smaller cages, whose conditions resemble the experimental setup.

Aims: We examined whether pre-experimental housing conditions influence song onset and production following transfer to acoustic recording chambers.

Materials and Methods: Using a double-blind design, canaries were housed for 90 days either in small cages (n = 11; 3–5 birds per cage) or in aviaries (n = 12; 6 birds per aviary). Birds were then transferred to individual recording chambers for an 8-day recording period. We quantified latency to song onset and daily song rate.

Results: Pre-experimental housing condition did not affect the day of song onset, with a median onset of day 2 in both groups (Wilcoxon's signed-rank test: $p = 0.25$). Across the experimental epoch, the daily average number of songs produced by aviary-housed birds was consistently equal to or greater than by cage-housed birds. Significant between-group differences were observed on two days (days 2 and 8), with aviary-housed birds producing more songs, and two additional days showed a similar trend (area-under-the-curve bootstrap test: $p < 0.05$ and $p < 0.1$, respectively).

Conclusions: These findings indicate that pre-experimental housing in aviaries does not impair song production following transfer to experimental conditions and may support higher song output. Our results have implications for experimental design, animal welfare, and optimization of neurophysiological data collection.

THE POWER OF STARTING SMALL – A WIN WIN SITUATION OF REFINEMENT AND SUSTAINABILITY

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The Central Animal Facility (CAF) at Hannover Medical School manages approximately 25,000 animals across various species, including mice, rats, gerbils, hamsters, guinea pigs, rabbits, Xenopus, sheep, pigs, and cats. These animals are housed under different hygiene standards, ranging from germ-free units to BSL 2 areas, utilizing both open-top cages and Individually Ventilated Cage systems. Large animals are housed in environments varying from farm like group settings to highly controlled Specific Pathogen-Free conditions. Because animal numbers at the CAF have halved over the past five to ten years, comprehensive restructuring of the housing for all species became possible.

The primary aim of this study was to implement a refinement and enrichment strategy tailored to the specific needs of each species, considering the facility's complexity, size, financial constraints, and sustainability. Over the last decade, a multi-faceted approach was adopted, focusing on species-specific solutions. Key interventions included introducing tunnel handling for mice, procuring 10,000 tunnels, and training personnel to use these tunnels for cage changing instead of traditional tail handling. For rats, Type IV cages were replaced with systems akin to those used for guinea pigs or rabbits. These are better suited to their behavioral needs and have, e.g., an increased height. Guinea pigs were transitioned to adapted rabbit stellages, while rabbits were transferred to floor housing. Pig pens were enlarged, incorporating rooting areas and ample straw to promote natural behavior.

Despite challenges like increased cleaning times and the need for adaptive strategies, these changes significantly enhanced animal welfare. Feedback loops between caretakers, researchers, and management enabled continuous adaptation, ensuring practical and effective solutions. The involvement of animal caretakers improved job satisfaction and integrated their insights into the refinement process.

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