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OA1M1 Genetic Engineering in Biomedical Research: Three Decades of in-vivo Models Culminating in CRISPR/Cas9 Technology

Jerchow, Boris, Presenting author
Max Delbrück Center for Molecular Medicine in the Helmholtz Association

Since almost three decades we are able to change the mouse genome at will. Since then, biomedical research has seen the advent of tens of thousands of new mouse models. Basic research and most preclinical studies have become unimaginable without Genetically Engineered Mice (GEM). Universities as well as academic and industrial research centers have built large and highly sophisticated facilities to keep and breed GEM and to ensure the reproducibility of their experiments.
In the early days of vertebrate genetic engineering we have mainly seen either lines overexpressing a randomly integrated transgene or the loss of function mutation of an endogenous gene resulting in a so called "knock-out" animal. Although the random integration of transgenes comes with the obvious disadvantage of variation with their locus of integration, over the years many meaningful models have significantly contributed to our understanding of biomedical questions.
In the mouse, "knock-out" mutant models can be generated via homologous recombination in embryonic stem (ES) cells. These loss of function mutants were key to today's understanding of gene function in a process known as reverse genetics. In the meantime, sophisticated methods of genome engineering in ES cells have been developed. Today, to name only some of the most common applications, we have mouse lines with complex mutations that mimic alterations found in patients, with genes that can be turned on and off, and lines that express tagged proteins, that can be easily visualized during embryonic development or in the progression of disease. Only recently, new ways to alter the genome have been found and for their ease of use have been quickly and broadly adopted by the scientific community. The new tools are targeted endonucleases, namely Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and others, but most importantly the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated 9 (CRISPR/Cas9) system. With the CRISPR/Cas9 system it is comparably easy to generate "knock-out" mutants via an endogenous repair mechanism termed Non-Homologous End Joining (NHEJ) and improvements are currently being developed to generate even complex targeted mutations via the alternative Homology Directed Repair (HDR) mechanism. Not only allow these new techniques to more efficiently alter the mouse genome, even most other species that were previously not amenable to genome editing can now be targeted. In my talk I will give an overview about the developments in genetic engineering over the last three decades with a focus on recent new technologies. I will try to set the stage for a discussion on what this may imply for research using genetically altered animals and how this might impact on animal numbers and the 3Rs approach.

OA1M2 Passenger mutations confound interpretation of all congenic knockout mice

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Targeted mutagenesis in mice is a powerful tool for the functional analysis of a gene. However, genetic variation between the embryonic stem (ES) cells used for targeting (previously almost exclusively 129-derived) and recipient strains (often C57BL/6J) typically results in congenic mice in which the targeted gene is flanked by ES cell-derived passenger DNA potentially containing mutations.
Comparative genomic analysis of 129 and C57BL/6J mouse strains revealed indels and single nucleotide polymorphisms that result in an alternative or aberrant amino acid sequence in 1084 genes in the 129 strain genome. Annotating these passenger mutations to the reported 129-derived full knockouts revealed that nearly all congenic knockout mice have multiple passenger mutations that potentially influence the phenotypic outcome. We illustrate this phenotypic interference of 129-derived passenger mutations with several case studies. A new Me-PaMuFind-It web tool will help researchers to estimate the number and effect of passenger mutations in their transgenic mice. Recently, more and more reports illustrate the impact of passenger mutations on the phenotypic outcome.

OA1M3 Epigenetics can influence fertility and health in offspring

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In nature, mammalian life is conceived inside the female genital tract, more specifically in the oviduct. Because the processes of fertilization and early preimplantation development take place in one of the most inaccessible parts of the mammalian body, it has been studied predominantly in vitro. In static culture platforms, the environmental conditions to which the gametes and embryos are exposed are in sharp contrast to what is observed in vivo. In the female reproductive tract, embryos are surrounded by a constantly changing minimum amount of media and are constantly moved by ciliated epithelia. The pre-implantation embryo, in vivo, develops in the absence of direct cell contact with the reproductive tract before implanting. It is free-floating, lacks a blood supply and is dependent on luminal secretions of the oviduct and uterus for its nutrition. The pre-implantation embryo expresses a number of receptors for signaling ligands. These signaling ligands are often paracrine factors, defined as factors that are secreted by one cell type and that execute their function on another cell type. They can originate from cells of the reproductive tract (e.g. cytokines) and have an effect on the embryo, or can be secreted by the embryo and have an effect on the oviduct or uterus. It is clear that these paracrine factors are crucial in the embryo-maternal dialogue. In vitro, the maternal genital tract is absent, but embryos do communicate with each other in group culture through autocrine secretions.
Only time will tell if epigenetic inheritance is an important contributor to animal variation and if it can be used to steer health, production and fertility in domestic animals across future generations. In the near future we may expect a great many articles, in domestic animal models as well as rodent species, on this tantalizing subject. The age-old argument, nature versus nurture, is no longer relevant. With the advent of epigenetics, the two are intertwined in all of us, and in all the animals we breed

OA1M4 Functional relevance of the non-coding genome in the establishment of the phenotype

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Josa S, Fernández A, Seruggia D, Cantero M, Fernández J, et al, Co-authors

Variations in the gene-coding regions have traditionally been used to explain the diverse phenotypes associated with different genetic backgrounds. However, the entire set of ~25,000 genes only accounts for ~2% of our genome. The remaining ~98% of our genomes corresponds to the non-coding part, largely containing DNA repetitive elements, retrotransposons, and, also, the DNA regulatory elements that genes require to be expressed specifically in time and space.
Studies of gene function in mice have been supported during the last decade by a nearly exhaustive collection of mutants, systematically obtained by homologous recombination in murine ES cells. The study of non-coding fraction of the genome, however, does not benefit of the same valuable resources. First, regulatory elements are usually found in the non-coding fraction of the genome, accounting for 98% of our genome and constitute a very large list of DNA sequences to be individually ablated genetically and, second, they are technically hard to target due to the nature of intergenic DNA, often populated by repeated sequences. At present there are new technologies enabling the functional assessment of the non-coding genome in a more systematic manner, using CRISPR-Cas9 strategies. This presentation will address some examples of CRISPR-mediated mutagenesis to efficiently inactivate non-coding regulatory elements in the mouse tyrosinase (Tyr) locus, which we had been studying extensively before in transgenes, hence in ectopic sites, although it was refractory to homologous recombination in mouse ES cell, to assess its function at the endogenous location. Mice lacking these non-coding regulatory elements show striking phenotypes, thus highlighting the relevance, in vivo, of DNA regulatory elements and their involvement in the establishment of the final phenotype of one animal.

OA2S1 Guiding Principles in Behavioural Laboratory Animal Science

Stanford, Clare, Presenting author
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The project to develop these Guidelines was launched by LASA.
Mindful of the need to recruit input from a broad range of professionals, whose expertise in this field is recognised internationally, LASA set up a steering panel that included representatives from other key Learned Societies (British Association for Psychopharmacology, British Neuroscience Association and the European Summer School in Whole Animal Pharmacology). In this talk, I will explain why LASA thought there was a need for these Guidelines, who they are intended to help, how we set about gathering and organising the information they should contain, and also offer a guided tour of the contents.

OA2S3 Effect of housing environment on the emotional state and welfare of laboratory mice

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Measures of emotional states in animals could be a valuable tool in the assessment of their welfare. A theoretical basis is that e.g. housing in different environments could affect their emotional state which in turn would influence how an animal perceive and respond in a short term situation (Mendl et al. 2009). The response of the animal could then be used as an indicator of its emotional state and consequently also of its welfare. This concept was investigated in mice in the present study.
Ninety female C57BL/6N mice were housed in three different housing treatments; barren (800 cm2 cage with bedding), standard (800 cm2 cage with bedding, cardboard house and nesting material) and enriched (2x1800 cm2 cages with two cardboard houses, two types of nesting material and two hammocks attached to the lids) with ad lib food and water, for 5 weeks. During these weeks the mice were exposed to two different behavioural tests – an exploration test and a contrast test (Wichman and Spangenberg, 2014) to measure their response in a short term situation. All handling of the mice was performed by letting the mice climb up on cardboard house turned upside down. An evaluation of the ease of handling was done on a three point scale at four time points during the study. In the exploration test mice (N=36) had to pass a push-door, with increased weight for each test day, to enter an arena containing different rooms and objects to explore for 5 min/day for a minimum of 9 days. The maximum weight pushed to reach the arena was noted as a measure of their motivation to explore, and exploration was measured as the number of entries to the rooms in the arena. In the contrast test the latency of the mice (N=54) to reach the goal area in a runway was registered. Half of the mice from each housing treatment received hazelnut crème (control) in the goal area during baseline training (BL) for 11 days (3 trials/day) and the other half received a regular food pellet (contrast group). To induce a positive contrast, the reward for the contrast group was shifted to hazelnut crème for the next 5 days (post-shift, PS). The main findings were that control mice in enriched housing had the shortest mean latency to reach the reward in the contrast test (P<0.05, for day 10 BL and day 1-4 PS). Within housing type there was a contrast effect day 1 PS in barren treatment, the contrast group had shorter latency than the control group (P=0.034). Mice from enriched housing visited more rooms the first day in the exploratory arena (P=0.027) but there was no significant difference between treatments in the maximum weight the mice pushed to enter the arena (P=0.552). The standard housed mice were classified as being the most easy to handle. In conclusion, the housing treatments affected the mice behaviour and mice in enriched housing showed a more active response in the tests which could possibly be interpreted as them having a more positive emotional state.

OA2S4 Curious and shy male rats behaviour in different environmental conditions

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Wild rats filogenetically adapted to the diversified environment. During their everyday life the rats interact with new elements of the environment. There are two different types of strategies based on the double effect caused by novelty curiosity to explore the objects and fear-harm avoidance. This is the basis of the domesticated rats' behaviour. It is difficult to take difference between the curiosity which results in active exploration and the animal who feels anxiety in a novel environment.
For categorization and determination of male rats' reaction to the new environment we planned a three steps examination. 53 male rats were used for observation. Glass box with open top was used as an open field arena. The floor was divided in 3x3 equal squares. There were three observation trials: 1st trial: Empty box without any objects. 2nd trial: Empty box, with another rat. Before the observation the observed and fellow rats were kept in the same plastic box as habitat. 3rd trial: Environment enriched box with a paper roll, a handful of paper chips and a red, plastic house. The animal was observed for 10 minutes in every trial. Horizontal and vertical movement, rearing, and the number and length of the grooming were counted in every trial. In the 2nd trial the social and in the 3rd trial the object interaction was counted. The rats were divided into two groups: inactive and active male rats. This is an arbitrary subdivision based on the number of the square crossing in 1-5 minutes of the first trial. If the square crossing number was less or equal than 20, rat was considered as inactive. There were significant difference in the 1st trial in vertical and horizontal moving between the active and inactive groups. The significant difference in activity disappeared in presence of another animal or an environmental enrichment. There was no difference in the number of grooming and in the social interaction. The most preferred object in the 3rd trial was the plastic house for both groups. Mainly there was no statistical difference in object interaction. In conclusion environmental enrichment reduced the anxiety in „shy" rats, the social interactions, novel objects erase the difference in rats' activity and behaviour. It is very important to use environmental enrichments for animals from the first minute because travelling and new environment can cause fear. If we cannot reduce the stress from the beginning of their arrival the anxiety cause alteration in their behaviour which can alterate the result of the research.

OA2S5 Effects of Reversing Light-Dark Cycle Following Transfer and Re-housing, on Behavioural and Physiological Parameters in Rats

Arts, Johanna, Presenting author
Envigo RMS BV

Rats are nocturnal animals, and many (behavioural) researchers choose to reverse the light-dark phases after transfer from the breeding facility to the research facility. It is not known whether this time shift in addition to transfer-stress results in a prolonged need for the animals to habituate to the new conditions. This study investigated the effects of such transfer-light regime reversal paradigm on plasma corticosterone and home cage behaviour in male and female Wistar rats.
Animals, Material and Methods Baseline measurements of body weight, plasma corticosterone, and home cage behaviour were collected from 48 male and 48 female Wistar Unilever rats (HsdCpb:WU, Envigo RMS B.V., The Netherlands) at the breeding facility after which all animals were transferred by road (truck and van) to a new facility. Control animals stayed on their before-transfer light regime, the experimental groups were housed on a (12 hr) reversed light regime at arrival. Measurements were continued at the new facility. Body weight measurements and CORT levels were analysed using a Restricted Maximum Likelihood approach using a linear mixed model in GenStat. For behavioural data we carried out Analyses of variance on the daily averages per cage. Results On average, transfer decreased body weight of rats by 3.0% in males and 3.2% in females. Plasma corticosterone in females increased after transfer. Active behaviour was decreased after transfer and recovered faster in the rats of both genders on a reversed light regime. Resting behaviour was increased after transfer and also recovered faster in the rats on a reversed light regime. The results of the behavioural observations show that animals housed under a reversed light regime after transfer acclimatize quicker than control animals. Discussion and Conclusions: In both sexes and treatment groups, activity was decreased after transfer. Both sexes showed a recovery in de reversed group after 1 week and a continuing decreased activity in the control group. Individual animals react differently to a complex stressor like external transfer (e.g. corticosterone levels). Using an outbred strain might have added to that variability. This study does not shift perspectives regarding acclimatization time needed after inter facility transfer of laboratory rodents. However, it does shed some more light on the effects of light regime reversal after transfer: We expected a light regime reversal to have an additional impact on the animals regarding acclimatization after transfer, but the results of the behavioural observations show that animals of which light regime was reversed upon arrival, acclimatize quicker than non-reversed control animals. Taking previous studies into account, we advise an acclimatization period of 2 weeks in males and female rats, regardless of whether a light regime reversal has been applied.

OA3S1 How to publish in Laboratory Animals, the official Journal of FELASA

Riederer, Beat M, Presenting author
Laboratory Animals Ltd c/o FBM, University of Lausanne

The international journal of laboratory animal science and welfare, Laboratory Animals publishes peer-reviewed original papers and reviews on all aspects of the use of animals in biomedical research. The journal promotes improvements in the welfare or well-being of the animals used with respect to the 3Rs, and in particular focuses on research that reduces the number of animals or which replaces animal models with in vitro alternatives.
This year the Journal celebrates its 50th anniversary. Novelties are: publication of 6 issues per year; abstracts are translated in French, German and Spanish; a News section for subscribing societies has been added for non-peer-reviewed contributions (in English or other languages). The Journal is published, on behalf of Laboratory Animals Ltd, by SAGE Publication Ltd. The impact factor 2014 is at 1,120, ranking at position 71/153 in Zoology and at position 53/133 in Veterinary Sciences. LAJ receives 200 submissions per year, with an acceptance rate of 34%. For the preparation of manuscripts, consult guidelines of LAJ for different types of manuscripts such as FELASA working party reports, reviews, full articles, short reports or letters to the editor (www.la.sagepub.com). Note that word counts are limited and depend on the type of manuscript. Use the Vancouver referencing system. Since two years LAJ has adopted the ARRIVE-guidelines (1) that help in the preparation of manuscript - like a check list. Give a precise and attractive title, sufficient background information, numerate objectives of the study, point-out major findings and indicate its novelty. Frequent observations are the lack of an ethical statement, or a missing justification how the number of used animals was calculated,

The guidelines lists also points that need to be mentioned and warrant reproducibility of the results. Studies should include both genders or a justification needs to be given when only one gender was used (2). By respecting the ARRIVE guidelines the quality of manuscripts is improved and it facilitates the work of the editorial board (3). Points that may lead to a decision of rejection or to major revisions needed, are that the research topic is marginal to the scope of the journal, bad study design or lacking description of methods, wrong animal models, lacking analgesia, wrong anaesthesia, insufficient statistical power, lacking novelty, linguistic problems, too wordy or exceeding word count. Considerable efforts are made to have a timely acceptable review process and fair and objective reviews. The overall goal is to increase the quality of manuscripts, improve the impact factor and visibility of the Journal.

OA3S2 Link between quality of reporting and evidence-based approach of systematic reviewing in animal research

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Ritskes, Merel[1], Co-Author

Publications are an important way of communicating scientific results. Details on the experiments described in these publications are needed to interpret the results, to be able to replicate the experiment and to use data for future research. Recent studies show that the quality of (reporting of) animal studies needs improvement.
Within clinical research the introduction of systematic reviews resulted in improved quality of (reporting of) clinical trials. In laboratory animal science the main driver for quality improvement has been to replace, reduce and refine animal experiments (3R). Evidence is accumulating that in particular the translation of animal research into clinical benefit is still subject to improvement (Landis et al., 2012), and the 3R framework is not going to solve that. Systematic review methodology is proposed as an effective methodology to achieve this and to increase the (translational) value of animal studies. Besides making transparent the quality and translatability of the animal studies, analysis of scientific literature through systematic reviews will provide new knowledge, better inform the ethical review of animal studies, improve the funding process, optimize the choice of animal model, increase the transparency of the translation of animal-based research, improve patient safety and help implement the Three Rs. A more effective search of literature will also prevent unnecessary duplication (Leenaars et al., 2012). Since the systematic review methodology was hardly known in animal research, we initiated its implementation in 2008. Since then SYRCLE (www.syrcle.nl) is dedicated to stimulate this evidence-based approach of systematic reviewing into animal research by: 1. developing tools and guidelines, 2. developing and providing education and training, and 3. Providing coaching during the process of performing systematic review of animal studies. To facilitate implementation of education on systematic reviews of animal studies in the Course on Laboratory Animal Science for researchers in the Netherlands we developed an e-learning module funded by the Ministry of Economic Affairs (responsible for the Law on animal experimentation). After completing the e-learning, the participants are aware of the potential of the methodology, its basic principles and its most important steps. Get an interactive introduction into systematic reviews of animal studies by following the free e-learning here: https://syrcle.ekphost.nl (registration code: syrcle).Conclusion: The evidence-based approach of systematic reviewing will make the quality of published animal studies transparent and, as a result, make clear that improvement of the reporting quality is essential in order to be able to ethically and scientifically justify animal studies.

OA3S3 Good Reporting Practice for Manuscripts in the Life, Health, Veterinary, and Agricultural Sciences

Bomzon, Arieh, Presenting author
ConsulWrite

Researchers must conduct their research honestly and ethically and a research report is tangible evidence that the research was done honestly and ethically. If research is not reported, it might as well not have been done. Assessment of reliability of a report is a necessary condition for the scientific process: the report might be read for planning a similar study in order to advance existing knowledge on a topic or to improve an existing treatment and management of a disease.
Science has become an increasingly global enterprise and the preferred language of almost all reports in peer-reviewed scientific journals is English. Since many of these reports are written by individuals who are not able to communicate fluently in English, a lot of reports are badly written. The common causes of badly written reports are a substandard quality of the report's language, which renders the report imprecise, inconsistent, verbose, and difficult to read and understand. Moreover, reading a badly written report has consequences: no advancement of knowledge, no improvement in existing treatment of patients, patient harm, and scientific misconduct. Writing an accurate, clear, concise, and coherent research report requires a communication strategy, a management plan, and guidelines for document development. Components of the communication strategy are defining the purpose of publication, assessing the audience (Who are the potential readers and what information does the reader need?), and establishing effectiveness criteria (How will the publication be used? What criteria should be used to judge the publication's effectiveness in achieving its purpose?). Components of the management plan are confirmation of resources, team assembly and briefing, integrating the schedule, establishing monitoring procedures, and having a document management system. Guidelines for document development have to be established or followed on content, structure, and navigability (editorial style sheet and instructions to authors). Research reports are rigidly structured narratives in four chapters, an Introduction, a Materials and Methods section, a Results section, and a Discussion (IMRaD structure). The Introduction outlines the background and rationale of the study in order to present the investigation's working hypothesis. The Materials and Methods section describes the investigation's size, design, setting, participants, study parameters, data sources, methods of measurement, and methods of analysing data, and contain ethics statements which confirm that all the experiments conformed to the relevant regulatory standards. The Results section presents the results of the observation and/or experiments, which could be descriptive or outcome data. The Discussion is a critical assessment of the entire investigation and ideally includes an interpretation of the results, a compare-and-contrast analysis, comments on the investigation's limitations, and conclusions.

OA3S4 Scientific writing in Laboratory Animal Science - Point-of view of a researcher

Jirkof, Paulin, Presenting author
Division of Surgical Research, University Hospital Zurich, University of Zurich

This talk deals with the characteristics and particularities of scientific writing in the field of laboratory animal science from a researcher's point of view.
Researchers working and writing in the field of laboratory animal science find themselves in very specific circumstances. First, the ethically and scientifically justified use of animals in research has several conditions, requirements and best practices per se. Additionally, not all researchers publishing in laboratory animal science journals, or journals in related fields, are full-time scientists. Many of them are some kind of service providers in animal based research settings, working in housing facilities or experimental units. The involvement of practitioners, comparable to some branches of veterinary or humane medicine or other applied sciences, has some obvious advantages like a realistic approach towards problems, awareness of significant needs of the field as well as a focus on feasibility of methods and on applied research questions. Nevertheless this may also come with several drawbacks like missing experience in scientific writing, time constraints or insufficient resources. In this talk, I will highlight the opportunities that research conducted by practitioners as well as full time laboratory animal scientists offer for better science involving animals and improved welfare of laboratory animals. I will give some comments on useful tools, study design, writing, review and publishing process and the dissemination of results from point of view of a young laboratory animal science professional.

OA4S1 Application of the 3Rs in regulatory toxicology

Robinson, Sally, Presenting author
AstraZeneca UK

It is a scientific, ethical and regulatory requirement that before any potential new medicine can be administered to man it must be investigated in animals in order to define safe human doses and reveal potential toxicity associated with the prospective medicine. While this requirement remains, it is essential to minimise the number of animals used and the adverse effects they may experience.
Since the objective of toxicology studies in animals is to identify potential adverse effects in humans, some of the animals used will suffer adverse effects. However, careful consideration and design of studies can reduce the impact on animals without compromising scientific goals or human safety and may indeed improve the quality of science. In this talk three evidence based examples of challenging status quo in study design will be shared. Firstly an older example regarding the removal of acute toxicity tests from the requirements for the development of new medicines. This case set a framework for the pharmaceutical industry to work together and share data to influence regulatory as well as practical change. The second example will showcase opportunities for micro-sampling in regulatory studies highlighting some of the potential challenges that have been overcome. The final example will focus on the data sharing that allowed refinement of the endpoint of maximum tolerated dose in early safety studies.

OA4S2 Comprehensive in vitro Proarrhythmia Assay (CiPA) testing paradigm: an opportunity to apply the 3Rs

Valentin, Jean-Pierre, Presenting author
UCB Biopharma sprl

While thorough QT (TQT) studies provide a robust estimate of drug-induced QT prolongation in normal subjects, they do not ascertain whether it is pro-arrhythmic (ProA) in normal subjects or patients. ICH S7B and E14 guidances have been effective in preventing new drugs from withdrawal due to unacceptable Torsades de Pointes (TdP) risk, but evidence suggests that not all drugs that block hERG elicit TdP, and that QT prolongation is highly sensitive but not very specific for predicting ProA risk.
It has been suggested that emphasis on hERG current assays as early screening assays likely contributed to the unwarranted attrition of drug candidates early during drug discovery, thereby negatively impacting evolving drug pipelines. The utility of the intense clinical focus on small changes in QT prolongation, which may not represent any significant pro-arrhythmic risk, has recently been challenged. A new, integrated non-clinical paradigm to assess the ProA risk of novel drug candidates in the absence of a TQT study has been discussed. This new paradigm, called "Comprehensive In Vitro Proarrhythmia Assay" (CiPA), aimed to evaluate ProA risk based upon a more comprehensive set of in vitro assays mechanistically linked to TdP along with more traditional in vivo QT studies. CiPA relies on data derived from human derived ionic currents, human-derived cardiomyocytes, and in silico reconstructions of human ventricular cellular electrophysiology to assess a compound's potential ProA liability. Specifically, the three components of CiPA are: (a) the in vitro evaluation of drug effects on individual human cardiac currents expressed in heterologous expression systems; (b) integrated, in silico reconstructions of ventricular activity as affected by measured effects on cardiac currents; and (c) confirmation of drug effects on human stem-cell derived cardiomyocytes, providing an integrated cellular response. Standardized protocols are being defined to interrogate drug effects on seven prominent cardiac currents involved in repolarization. The O'Hara-Rudy computer reconstruction is one candidate in silico model expected to integrate drug responses on multiple cardiac channels and detect emergent ProA events, including early after polarizations. Finally, human stem cell-derived cardiomyocytes will provide cell-based integrated electrophysiological responses used to assess the veracity of in silico reconstructions. Such studies may involve microelectrode arrays, voltage-sensitive dyes, or traditional, but lower throughput, transmembrane potential recordings (using microelectrode or perforated patch techniques).Those proposed approaches should supersede the need for animals based action potential duration assays (e.g., APD in canine Purkinje fibre) and in vivo ECG assessment (e.g., QTc in guinea pigs, dogs and/or primates), whilst increasing the likelihood of success of drug candidates.

OA4S3 Animal-free methods for better understanding toxicity in humans

Roggen, Erwin, Presenting author
3Rs Management and Consulting ApS, Lyngby, Denmark

Animal testing has been, and still is, the corner stone of risk assessment for all industry sectors, but the Cosmetic sector. While the last 2 decades have shown the emergence of partial replacement or refinement/reduction methods as part of comprehensive testing strategies in order to reduce the number of animal tests, animal testing yet not be completely replaced.
Technological development has made it possible to perform mode-of-action and mechanistic studies providing detailed understanding of the adverse pathways leading towards toxicity, and the identification of the key events which in concert lead to the toxicity endpoint under investigation. It is believed that test methods addressing these key events can be integrated in testing and assessment strategies providing relevant

information about the safety in humans of an ingredient or a product. This is exemplified by the test methods and strategies emerging in areas like sensitization and genotoxicity. The success of the scientific approach is shown by increasing evidence that some of the individual test methods and strategies are more accurate in determining human toxicants than the currently applied animal models.
Thus, increasing mechanistic understanding has resulted in tools that are useful for addressing direct efficacy and safety questions, and thereby reducing animal testing.

OA5W1 Enhancing reproducibility of in vivo preclinical research: The Innovative Medicines Initiative Collaborative model

Vaudano, Elisabetta, Presenting author
Innovative Medicines Initiative

The Innovative Medicines Initiative (IMI) is a public–private partnership (PPP) between the European Commission and the members of the European Federation of Pharmaceutical Industries and Associations (EFPIA). The IMI's key mission is to enhance the competitiveness of the pharmaceutical sector in Europe, for the benefit of patients and scientists, by supporting open innovation and pre-competitive research in pharmaceutical research and development (R&D).
The use of animals in research and testing is a highly sensitive, yet vital part of the long and complex process involved in the creation of new medicines. While the insoluble problems of species differences and animal-to-human extrapolation inevitably limit the value of animal studies for the prediction of the actions of drugs in humans and a number of major technological developments have recently opened up possibilities for more-direct, human-based approaches, it is clear that a key concern that plagues the area is that of the reproducibility and relevance (e.g., use of models with good predictive and construct validity, proper choice of statistical analysis) of its research findings. For drug development, robust and reproducible data are key drivers for decision making, determining patent strength, time-to-market and consequently availability of new treatments to patients. PPPs like IMI create unique opportunities to join efforts among all relevant stakeholders to tackle together these issues and to open new avenues towards rationalising the use of animals in biomedical research, by focusing on validated reproducible models directly pertinent to drug action in human patients. In my talk I will provide some example from IMI projects that are contributing to an improvement in the data quality of pre-clinical in vivo studies via the delivery of reliable and reproducible models with harmonized and standardized protocols and procedures. I will touch on the importance of open collaboration and sound data and knowledge management as a pillar for success. I will also show how this can lead to a significant contribution to the 3Rs (replacement, reduction and refinement) in the use of experimental animals in preclinical research. Finally, I will present some relevant upcoming initiatives.

OA5W2 The value of animal models and their limitations: The minipig

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For animal studies in toxicology or ADME (absorption, distribution, metabolism and elimination), typically a rodent and a non-rodent species are used. As a non-rodent species the dog, the non-human primate (NHP) and also the minipig is available. The selection of the non-rodent species should always be based on good scientific rationale. The extrapolation of animal data to humans is best when the most "relevant" species is used for the study.
The choice of the non-rodent species is mainly driven by selecting one species that expresses the pharmacological target. Historically, the dog was used by default and if not suitable, the non-human primate (NHP) was an alternative. For biotherapeutics the NHP is often chosen based on genetic homology with humans. But physiological differences, limitations in translation to humans and ethical concerns have led to the search for alternative models. Anatomical, physiological and biochemical similarities between pigs and humans suggest the minipig is a useful model in safety testing and ADME of new chemical or biological entities. Cardiovascular, urinary, ocular and digestive systems and the skin are demonstrated as translational to human. Minipigs are larger and grow faster than dogs and become sexually mature earlier. From a preclinical toxicology perspective, this facilitates that mature animals are used in early studies for drug candidate selection when compared with dogs. In contrast to the dog and NHP, minipigs can easily be used in reproduction and developmental toxicity studies (embryo-foetal studies and juvenile toxicity studies).Other important factors make the pig a good animal model: it is bred to high quality standards, is well understood, the generated data are reproducible, and it is readily available. Differences between domestic farm and miniature breeds relate to growth rate and size at sexual maturity rather than actual anatomic differences in organs and structures. Minipigs require less space and diet, are easier to handle, and require a lesser amount of the testing compound. The minipig genome has been sequenced. This information can be used systematically in the selection of the non-rodent species for nonclinical safety studies and has been the basis for its use as animal model in general. Minipigs reveal enzyme expressions and activities regarding P450 enzymes similar to humans and thus show similar metabolic pathways of xenobiotics. We are currently investigating non-P450 related enzymatic pathways in the minipig. While every model has its limitations, the minipig provides significant advantages for the biomedical community owing to physiological similarities to humans, growing background data and regulatory acceptance. Recent advancements in generating transgenic minipigs is expected to expand its future use. Overall, the use of minipigs for the generation of human-relevant safety data will minimize any unnecessary use of dogs or non-human primates.

OA5W3 Animals in Basic Research: Modeling Human Congenital Malformations in Mice

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Congenital malformations or birth defects are caused by altered embryonic development. The underlying causes can be spontaneous or inherited genetic mutations or teratogenic effects. As mouse embryonic development is strikingly similar to humans, its analysis is providing important insights into normal and aberrant human development. Therefore, the mouse is the best animal model to study the molecular alterations underlying human congenital malformations.
Birth defects are rather common as about 1 in 50 babies are born with genetic anomalies and may result in death of the embryo within the first weeks of gestation. Many of the congenital malformations are quite severe as birth defects are the cause underlying about 20% of infant mortality. These birth defects are most often either structural, i.e. alter the skeletal or organ (e.g. heart, kidney, brain) morphology, functions or physiology (e.g. hormone production, metabolism disorders). The majority of these congenital malformations arise during human embryogenesis, i.e. the first two months of human gestation, and molecular analysis has shown that the molecular mechanisms governing organo- and skeletogenesis are evolutionary highly conserved. The mouse is the smallest mammal whose embryonic development closely resembles the one of humans. In addition to genetic mutations, teratogens (environmental or drug substances) can also cause birth defects by interfering with normal human

embryonic development. Human congenital malformations of genetic origin are caused by an immense variety of genome alterations. Mutations range from point mutations to large duplications or deletions that either affect coding regions or gene regulatory regions. Using reverse molecular genetics and genome editing an ever increasing number of mouse models for human congenital malformations are being generated, which provide insights into the normal and altered molecular mechanisms. However not all human congenital malformations can be easily modelled in mice as not in all cases the underlying mutations and alterations have been identified or are well understood. In particular, congenital disease resulting in complex mental illness cannot always be modelled in mice. Also congenital malformations without a clear genetic origin are often difficult or impossible to precisely model in mice. Finally, foetal development in mice is very short, while it is very long in humans. Nevertheless, genetically altered mouse model has uncovered the complex molecular networks underlying normal and aberrant embryonic development, which has tremendously helped the understanding of congenital malformations. These studies continue to provide important insights into the often complex genomic re-arrangements that are causal to the developmental alterations that result in birth defects.

OA6S1 Data sharing: Convert challenges in opportunities

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Today, research, industry and government agencies generate huge amounts of data. In five years, these data can grow in 650%. Many of these data are from animal experiments.
How can we make the most out of the available data and, at the same time, comply with the 3-R principle of animal testing (Replacement-Reduction-Refinement)? One possible way of tackling both issues at once, is to share the data from those experiments with the relevant communities. However, this is not a trivial issue. In fact, data sharing implies planning and deciding, first, why share the data, and then how, where and with whom. In this talk, we will explore the theme of data sharing by examining the specific relevant questions behind it. We will take the example of an imaginary scientist who is planning her/his next animal experiment and which steps s/he takes to share the resulting data. We will explore the arguments behind the question of sharing data or not, from the altruistic view to the selfish one. Then, we will go through the different possible ways of data sharing, e.g., share in public repositories or in private ones?, publish in a journal or in the lab website?, or even, share with the world or with a closed community? We will also discuss how metadata makes data FAIR (Findable; accessible; interoperable; reusable) and machine readable. Further, we will also discuss the licensing issue of data sharing. Data sharing is a big challenge from a multidisciplinary perspective and offers, among others, the opportunity of generating more knowledge from less data.

OA6S2 3Rs databases: a shared responsibility for the common cause?

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According to the European Directive 2010/63, researchers are obliged to consider the 3Rs when designing and performing procedures involving animals. To accomplish this, the latest information about 3Rs-methods has to be identified.
Databases and informative websites can facilitate retrieval of specific 3Rs related information for scientists, but also for the institutional Animal Welfare Bodies, project evaluators (animal ethics committees) and the Competent Authorities. These databases and websites save time-consuming searches, facilitate completeness and contribute to the 3Rs. To be successful, they should be easily found, accessed, managed and updated. In addition, relevant data should be easily retrieved.
Generally, only the establishment of this kind of databases is supported by external funding sources. Most databases that are freely accessible cannot be regularly updated, let alone expanded, due to a lack of further support. In this lecture we will explore and discuss the opportunities these information sources offer and the challenges they face with respect to maintenance, based on our experiences with the 3Rs Database Programme of the 3Rs-Centre Utrecht Life Sciences (ULS).
Currently, the 3Rs Database Programme contains the Humane Endpoints website and Interspecies Database. In order to maintain and update these information sources, an annual budget of approximately 150.000 euro's is needed. Although the programme serves the common good, financing it seems not to be seen as a shared responsibility. 'Who should pay for activities like this database programme?' and 'What challenges does the 3Rs-Centre ULS face in fund raising?' are central questions which will be discussed.

OA6S3 Randomised block (RB) experimental designs should replace completely randomised (CR) designs in animal research

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Many animal experiments are producing false positive results. Begley and Ellis (2012) were only able to reproduce the results of 6/53 "landmark" papers in cancer research. Similarly Scott et al (2008) using an improved design re-screened >50 drugs which apparently prolonged lifespan in a murine model of ALS but which were ineffective in humans. None of them were effective. The cost of such false positive results may amount to $25 billion annually in the USA alone (Freedman et al 2015).
The case for change: One, among several possible explanations for all these false positive results, is that scientists are using the wrong experimental design. As long ago as 1992 a survey found that completely randomised (CR) designs were much more widely used than randomised block (RB) designs in work involving laboratory animals (Festing 1992). Although there does not seem to have been a more recent formal survey, observation shows the situation has not changed significantly since then. Most agricultural and industrial research is done using randomised block (RB) designs. In these designs the experiment is split up into a number of "mini-experiments" or "blocks" each of which has a single experimental unit (often an animal or cage of animals) on each treatment. Blocks are separated in time and/or space with randomisation being done separately for each block. The outcomes for each block are then combined using a two-way analysis of variance without interaction. Because each block in a RB design has only a few experimental units, these can be closely matched in time and space, leading to increased power. The separate randomisation of each block also reduces the chance of bias due to unlucky or incorrect randomisation in a CR design. RB designs are also logistically more convenient as each block is a separate "mini-experiment" so they can be set up and processed at a different times and/or in a different locations (Festing, 2014). The current widespread use of CR designs in laboratory animal work may be because scientists have copied clinical trials where RB designs are rarely appropriate, and because of a lack of statistical training among research scientists. Conclusions Laboratory animal experi-

ments are much more like agricultural experiments, where there is good control of the animals and their environment, than clinical trials where large simple experiments are needed due to inter-individual variability. The advantages of RB designs are overwhelming and need to be brought to the attention of scientists using laboratory animals. They should be strongly encouraged to use them in all their experiments.

OA7M1 The mouse gut microbiome revisited: from complex diversity to model ecosystems

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Laboratory mice are the most commonly used animal model in translational medical research. In recent years, the impact of the gut microbiota (i.e. communities of microorganisms in the intestine) on host physiology and the onset of diseases, including metabolic and neuronal disorders, cancers, gastrointestinal infections and chronic inflammation, became a focal point of interest.
There is abundant evidence that mouse phenotypes in disease models vary greatly between animal facilities or commercial providers, and that this variation is associated with differences in the microbiota. Hence, there is a clear discrepancy between the widespread use of mouse models in research and the patchwork knowledge on the mouse gut microbiome. In the presentation, I will summarize data pertaining to the diversity and functions of the mouse gut microbiota, review existing work on gnotobiotic mouse models, and discuss challenges and opportunities for current and future research in the field.

OA7M2 The impact of diet and gut microbiota modulation on disease expression in experimental animal models

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During the last decade it has become evident that the gut microbiota (GM) plays a pivotal role in human health and disease, with GM dysbiosis being implicated in the development of many diseases ranging from autoimmune diseases like asthma, over obesity to probably also influencing behaviour and mood. Similarly, the GM strongly influences experimental animal models for studying such diseases. Diet is one of the key factors influencing GM and thus also experimental animal disease model outcome.
The mammalian gut is inhabited by trillions of microorganisms representing all 3 kingdoms of life and hundreds of different species. This complex microbial society is referred to as the gut microbiota (GM). During the last 10 years it has been shown that GM dysbiosis is implicated in the development of a wide range of human diseases – and using experimental animal models casual relationships between either specific GM members or GM patterns and certain diseases (obesity, oedematous severe acute malnutrition and asthma, to mention a few) have been established. The GM is a key player in immune system development and maturation in humans as well as animals. This means that early life GM changes will have long term effects on the host – and consequently also affect disease expression in experimental animal disease models. However, also later in life, it is indeed possible (and often quite easy) to manipulate GM in a direction influencing the host phenotype – and thus also experimental animal model disease expression. Consequently there is great interest in being able to control and ideally also manipulate the GM of experimental animal disease models in a desired direction leading to e.g. a stronger, weaker or more uniform disease expression pattern. One way of doing this is via dietary modulation actively using the feeding strategy to "push" the GM in a desired direction. We have for instance showed how a diet devoid of gluten containing (cereal) ingredients compared to a conventional chow diet significantly lower diabetes incidence in the Non-Obese Diabetic (NOD) mice. Similarly, the by varying other macronutrients the abundance of specific GM members can be manipulated. Also micronutrients like some minerals (e.g. zing) and vitamins (e.g. vitamin D) significantly influence GM and disease expression in certain animal disease models. Even differences in drinking water pH have been found to profoundly influence diabetes incidence in NOD mice. In the present talk illustrative examples of how different diets/feeding strategies can be used to manipulate GM, immune system development and disease expression in experimental animal disease models will be discussed.

OA7M3 Sensitivity to oxazolone induced dermatitis can be transferred with the gut microbiota between mice

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Atopic dermatitis (AD) is a skin disease associated with the gut microbiota (GM)[1]. If the GM has a substantial impact on disease expression and variation, animal studies may be made more cost efficient if mice are inoculated with a GM inducing a strong expression of AD, because effect size may become increased and inter-individual variation may be decreased. The aim of this study was therefore to investigate if high and low sensitivity to dermatitis can be transferred with the GM between mice.
Methods: Oxazolone-induced dermatitis was applied on the ear of barrier-bred mice and based upon evaluation of the clinical score, ear thickness, and the concentrations of pro-inflammatory cytokines, a high and a low disease responder were selected as donors. Faeces from the donors was given to two groups of pregnant germ-free dams, and subsequently the off-spring of these as well as a third germ-free group was used for induction of dermatitis. The degree of dermatitis was compared between the three groups and the GM of the mice receiving a high and low responding microbiota was analysed by high throughput sequencing in order to identify bacterial taxa associated with the disease. Results: It was possible to transfer the AD phenotype with the GM from barrier-bred mice to germ-free mice. The mice inoculated with the high responding GM had a higher clinical score, increased ear thickness, and increased levels of IL-1β, TNFα, IL-4, IL-5, and IL-6 compared to recipient mice of the low responding GM. Germ-free mice exhibited high disease response in all parameters. The high responding group had more abundance of species in the GM belonging to the family of Lachnospiraceae and Rikenellaceae, as well as Bacteroides uniformis. The GM diversity was lowest in the mice colonized with the low responding microbiota due to absence of a number of species belonging to the order of Clostridiales. Discussion and conclusions: The high and low responding phenotypes were clearly transferred to germ-free mice. The low GM diversity in the mice colonized with the low responding microbiota, together with the high disease respond in the germ-free mice, indicate that the GM impact on AD in mice is a question of presence of protective bacteria rather than a question of diversity. Sequencing of the GM did not clearly show any protective bacteria evident in the low responding group compared to the high responding group, although there was a tendency that the low responding group had greater abundance of unclassified species of Lactobacillus as well as Bacteroides. Both of these species has earlier shown to have anti-inflammatory effects [1-3]. This study underlines that the GM has a strong impact on AD in mouse models, and that the power of studies may be increased by the application of mice inoculated with a specific GM from high responders due to the increase in effect size. This increase in power can be used to reduce the number of mice used in oxazolone-induced dermatitis study designs.

OA7M4 The impact of the microbial diversity on the colitis phenotype in a bone marrow chimera model

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The interleukin-10-deficient mouse (Il10-/-) model is well suited to model Crohn's disease. The genetic background as well as the microflora influence the severity and the development of colitis. Cdcs1, a region on the murine chromosome 3, was identified as a modifier for colitis susceptibility. B6.129P2/J-Il10tm1Cgn/Ztm (B6-Il10-/-) are partially resistant, whereas B6.Cg-Il10tm1CgnMMU3(D3Mit11-D3Mit348)/JZtm (BC-R3), a congenic strain, is susceptible for colitis.
The aim of this study was to investigate the genetic influence of Cdcs1 on the colitogenic potential of hematopoietic cells. Materials and Methods: B6-Il10-/-and BC-R3 was genotyped with an array consisting of 150 000 single nucleotide polymorphisms (SNP). First, an acute graft versus host (GvH) reaction between the two strains was excluded with a mixed lymphocyte culture (MLC).Second, bone marrow chimera experiments between B6-Il10-/- and BC-R3 were performed with animals housed conventionally or under specific pathogen free conditions (SPF) according to FELASA guidelines. Bone marrow from B6-Il10-/- and BC-R3 was used to create chimeras on B6-Il10-/- and on BC-R3 background. Magnetic resonance imaging (MRI) as an in vivo tool and histological scoring of the colon were performed characterizing the colitis. The microflora of the cecum between the two hygienic areas was compared. Results: Apart from the Cdcs1 congenic fragment in BC-R3, both strains were highly similar in their genetic background and no GvH reaction was detected in the MLC. Bone marrow chimera experiments, performed with mice housed under conventional conditions and using MRI and histological scoring of the colon, revealed a high variation within the groups. Thus, differences between the strains were not detectable anymore. Next the influence of the microbiota was minimized using animals housed under SPF conditions to analyse the impact of haematopoietic cells within the model. Indeed, the variability in the groups decreased and an effect of Cdcs1 from the hematopoietic cells was visible. As expected, animals which received bone marrow from BC-R3 showed an increased colitis scoring compared to B6-Il10-/- recipients. The microflora analysis between the two housing areas was characterized and differences were identified. Discussion and Conclusion: The Cdcs1 region in the BC-R3 animals has an important impact on the induction of colitis and mainly the hematopoietic cells seem to trigger inflammation. Furthermore, we excluded the possibility of a GvH in this model. Thus, we show that especially the microflora but also the genetic background influence the pathogenesis of colitis in bone marrow chimera experiments.

OA7M5 Intestinal immune response of human microbiota-colonized C57BL/6 mice differs from that of mice colonized with a murine microbiota

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Introduction: Human microbiota-colonized mice are widely used in microbiome research. Though the approach has been effective in linking certain enterotypes or organisms to an impaired health state, human microbiota-colonized mouse models are reported to have important limitations, such as lower colonization rate than murine microbiota-colonized mice and poor priming of the immune system (1). The aim of this project was to characterize these limitations in the C57BL/6 model.
Materials and methods: Germ-free C57BL/6NTac mice were colonized with a human or murine gut microbiota and housed in gnotobiotic isolators. Mice were bred allowing for the microbiota to be passed on to the offspring. The V3 region of the 16S rRNA gene in fecal pellets from parents and offspring was sequenced on an Ion Torrent PGMTM system. Gene expression of immune cell markers was measured in colon and ileum using a TaqMan® assay. Results: The cytotoxic T cell marker CD8 had a lower expression in ileum and colon of the human microbiota-colonized mice (ileum: p<0.0001; colon: p<0.05), which was comparable to germ-free control mice. Likewise, the T helper cell marker CD4 was lower in ileum (p<0.05), but not in colon. The regulatory T cell marker FOXP3 was lower in ileum (p<0.05), while there were no differences in the dendritic cell marker Itgax. Interestingly, there were no differences between the parent and offspring generations for any of the markers. Discussion and conclusions: A certain composition or the presence of specific bacteria is necessary for stimulating the gut immune system in the same way as mice colonized with a murine microbiota, i.e. a microbiota adapted to laboratory mice. It has been proposed that a host-specific microbiota is necessary for optimal immune priming (1), but as the microbiota of laboratory mice is considerably different from that of wild mice (2) and expected to primarily derive from human caretakers, it can also simply be linked to the presence of certain bacteria independent of host-specificity. Regardless, impaired immune priming of human microbiota-colonized mice is an important, yet often neglected, consideration, which may weaken the translational value of microbiota-humanized mouse models targeting inflammatory and immunological pathways, unless accounted for e.g. through the diet or refined colonization procedures. The work was funded by Taconic Biosciences and Innovation Fund Denmark. The project is a collaboration between Taconic Biosciences, University of Copenhagen and the 3G Centre (Gut, Grain and Greens).

OA7M6 Luminal and wall-adhered gut commensal microbiota changes after Salmonella enterica serovar Typhimurium LT2 inoculation in rats

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Gut Commensal microbiota (GCM) plays a key role in immune system development and acts as a protective mechanism against infections. Poor diversity in GCM is described in inflammatory disorders. Indeed, a limited diversity may elicit an increased susceptibility to intestinal inflammation. We previously observed that GCM from healthy rats varies significantly depending on the commercial origin. Our aim is to describe GCM after oral Salmonella inoculation in rats from different commercial breeders.

Materials and methods: 12 specific-pathogen free (SPF) Sprague Dawley male rats from 3 different vendors (A, B and C) were used (n=36). Rats from vendors A and B were derived from many generations of harbouring altered Schaedler flora (ASF) that was once inoculated to the founders; whilst rats from vendor C were derived directly from germ-free animals that received ASF. Rats were orally inoculated with 1 ml (108 ufc/ml) of the Enterobacteria specie Salmonella spp. or 1 ml of saline solution and euthanized 24h after. Luminal and epithelial-adhered GCM of ileum and colonic samples were characterized by high-throughput DNA sequencing and fluorescent in situ hybridization (FISH) respectively. Results: As expected, rats from vendors A and B presented a higher GCM abundance. Particularly, these animals showed a significant amount of Lactobacillus spp and Bifidobacterium spp both in the lumen and, to less extend, adhered to the intestinal wall. These bacteria groups were nearly absent in the intestine of rats from vendor C. Rats from vendor C did not present bacteria of any group adhered to the ileum wall. Enterobacteria were poorly represented in the lumen and they were only adhered to the ileum wall in vendor C. Salmonella spp. infection showed a tendency to favour bacterial adherence in all the groups. However, this effect was particularly relevant in the ileum of vendor C rats. Similar changes were observed in the colon. Discussion and conclusions: Animals with more diverse bacterial population did not present adhesion of Enterobacteria. The less diverse epithelial GCM of vendor C might encourage Enterobacteria to gain unoccupied bacterial niches. These results might indicate that the intestinal immune system of animals with a lower GCM abundance triggered a different activation of innate immunity than those bred under a richer microbiological environment. It is necessary to correlate these results with the study of the immunological response in these animals, but, so far, our data suggest that when selecting an animal model where microbiota is relevant, a specific microbiota characterization is essential. Vendors should encourage characterization of microbiome and promote maintenance of the microbiota profile.

OA8S1 Improving translational qualification of Discovery R&D animal studies

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Lack of animal model translatability has been postulated as a contributing factor to the attrition problem facing the pharmaceutical industry. We describe a strategic approach to assess animal model quality to tackle preclinical-clinical concordance. Improved translational relevance of animal models should positively impact attrition rate and application of robust translational models could impact cycle time and decrease animal numbers (less animals used in developing/refining low value models).
Attrition rates of Phase II clinical trials remain high with the majority of failures due to lack of efficacy (Arrowsmith and Miller, 2013). Animal models are one area of potential contribution to drug candidate attrition, with concerns raised about lack of translatability and weak methodological rigour (see for example Cressey, 2015). At GSK we are driving a strategic approach to the use of animal models through the formation of multi-disciplinary animal modelling strategy teams in parallel with a QBA (question-based-assessment) tool. Animal Modelling Strategy Teams (AMST's) consist of multi-disciplinary experts with the composition designed for the specific scientific question/hypothesis. Such a multi-disciplinary approach allows the breadth of internal capability, expertise and previous experience to be leveraged. The addition of a clinician within such modelling discussions has proven to be paramount to retain line of sight to the patient. The team aims to understand the biological pathways underlying the disease and relate to the biological relevance of a given animal model. In support of the AMST, and often as an enabler for the review, a QBA tool has been designed; the Animal Model Quality Assessment Tool. The AMQA is a structured approach to defining the translational relevance of an animal model for a specific question. It can be applied to both mechanistic and disease models and gives a qualitative assessment of the model. The approach highlights aligned biology, whilst weaknesses can be identified and refined, or ultimately different modelling approaches can be highlighted as future investment areas. Additionally, areas of contention can be identified to facilitate the informed use of assay results by decision makers. Learnings from AMST discussions will be presented, along with a worked example of the AMQA tool. We aim to not only ensure the judicious use of animals, but optimise translational relevance and recognise weaknesses in our animal models. This forms part of the animal research strategy at GSK.

OA8S2 The translation-standardisation paradox: on the added-value of translational strategies

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A growing number of publications (1,2) show that the translation of animal experimental data into clinical use is low. Several reasons are suggested for this translation problem: failures in methodology (e.g., not applying blinding and correct methods of randomisation), not performing systematic reviews before planning animal studies and a lack of attention to species differences between human and animal (3). In this paper we focus on this last reason.
Flaws in translation often start in the lack of the acknowledgment that "An experimental animal is not a patient". The differences between the standardised experimental animal and the complex patient are enormous. The justification for using in vivo instead of in vitro methods is often to mimic the complexity of the patient as a complex organism. But current scientific practice prescribes a standardised animal under standardised conditions, leading to a reduction in complexity! This trend to standardisation can frustrate the chances for a successful translation to patients. We call this the standardisation-translation paradox. How to tackle this paradox? The challenge is to incorporate the complexity of the patient into the research. The inclusion of complexity is possible by (a) using more than one inbred strain, using both sexes and animals of different age and species, (b) developing more disease models for one disease could be used or a disease models that are more complex, e.g. a model that combines hypertension, kidney and heart problems. This idea is not new, but combined models that are evidence based and start from the patients' perspective are still rare, (c) incorporating the patient into the research, e.g. using humanised mouse or human biopsies and patient based data. The future lies in combining the patient and animal research into one research project in which the translation from patient to the animal is the starting point, rather than the translation from animal to patient. New techniques, like micro-dosing, big data on enzymes and genetics, make enable to start with the patient and then translate the questions arising from these data into relevant animal models. Conclusion A way to address the problem of low translatability is the development of "translational strategies". These involve implementing better methodology (e.g. systematic reviews, blinding, better randomisation) and integrating the research in order to incorporate patient data into the research practice.
The development of translational strategies is funded by the Netherlands Organisation for Scientific Research (NWO_313-99-310)

OA8S3 Aquatic models – The "world" of aquatic animal models behind zebrafish

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Zebrafish has been the main aquatic species used in research for the last decade. The use of zebrafish has been increasing exponentially and started to appear frequently in high rated publications. The successful use of zebrafish in relevant human research areas allowed this species to be seen as one of the most promising laboratory animal models in the foresight years. Nevertheless, the aquatic environment hides many more species that can play an important role in laboratory sciences.
Rodents, due to their evolutionary proximity to humans, will always be a first choice as laboratory animal models; however, there are some aquatic species that have proved to be excellent laboratory models (as a first choice), or even, good alternatives to the models used nowadays. Cancer, brain and heart diseases, tissue regeneration, aging and evolution are research areas that benefited substantially from the use of aquatic species as biological models. At the same time, when considering the 3 R's (reduction, refinement and replacement), fish are basal in the vertebrate evolutionary scale (although sharing many signalling pathways with mammals), which make them a very interesting alternative animal model. Beyond Zebrafish (Danio rerio), species like Japanese Medaka (Oryzias latipes), Pufferfish (Tetraodon nigroviridis) and Guppy (Poecilia reticulata) are used as model fish species. Their importance has been growing because they showed to be valuable laboratory animal models to study "human-related" topics. Interestingly, their genome shows remarkable similarities to the human genome (despite their ancestral tetraploid origin), which allow researchers to use them in evolutionary studies or to clarify the mechanisms underlying certain human diseases (for example, Huntington's and Alzheimer's). Fish are the largest Vertebrate group, with around 32 000 species described. Also, we can find that other aquatic animals have proved to be excellent laboratory models in different (important) research areas, such as: the axolotl (Ambystoma mexicanum) and the frog (Xenopus laevis) (Amphibia); the horseshoe crab (Limulus polyphemus) and the freshwater crab Paratelphusa hydrodromous (Crustacea); or the amphioxus (Branchiostoma lanceolatum) (Cephalochordata). Aquatic models can also be very useful in other research fields, such as: Aquaculture, Ecotoxicology, Marine pollution, Aquatic Biodiversity, Climatic Changes (through population studies), Fisheries, Animal Behaviour, among others. When deciding which laboratory animal model to use in their experiments, all researchers should consider the potential of the large number of aquatic species available.

OA8S4 Challenges for modelling traumatic injury: from Bench to Bedside and Back

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Trauma is responsible for a large proportion of the world's burden of disease, and is by far the biggest killer of young adults. Beyond mortality, outcomes for critically injured patients remain poor. Haemorrhage is the leading cause of preventable death and its effects are directly correlated with the incidence multi-organ failure in survivors. Trauma research is challenging due to patient heterogeneity, limited randomised controlled trials and poor in vitro modelling of systemic injury response.
Preclinical research remains a necessary adjust for mechanistic understanding of traumatic injury. Insistence of models that require an accurately scale version of the clinical scenario is misguiding and the development of a one-fits-all model is not a feasible approach. In fact it is likely that efficacy studies may require testing in different species and model of injuries. Therefore is important to understand the purpose of a given model and the criteria by which the experimental readouts will be clinically relevant. It also poses important experimental and welfare challenges associated to modelling cause and timing of injury and prehospital and intra-operative care; the limited inter-species validation of coagulation profile; the use of anaesthesia/analgesia, and how to sustain intensive care. Our preclinical unit at the Centre for Trauma Sciences remains instrumental for interdisciplinary discovery and translational research. We will address the abovementioned challenges and present our ongoing refinement strategies promoting the implementation of minimally invasive functional coagulation assays to study coagulopathy and imaging approaches to monitor cardiac function. The use of rotational thromboelastometry (ROTEM®) provides a simple and rapid evaluation of clot dynamics in whole blood for the rapid identification of acute traumatic coagulopathy in our trauma models (~15% reduction of mean clotting firmness from baseline), and have proven of greater value than other coagulation tests (PT, aPTT) in diagnosing and managing trauma haemorrhage. We have also implemented the use of echocardiography to assess trauma-induced secondary cardiac injury which may result in cardiac dysfunction. Changes in stroke volume and cardiac output (~65% and ~20% decrease to baseline, respectively) after trauma/bleeding insult are correlated with data on blood-derived cardiomyocyte injury biomarkers. These tests can be performed as point of care, facilitates real-time monitoring of organ function during the bleeding /injury phase and the fast availability of results may assist clinical decisions on need for transfusion. The implementation of such clinically relevant readouts improves the translation efficacy of our trauma models and its impact on trauma clinical management.

OA9M2 Imaging neuroinflammation in neurotrauma models: impact on translational studies

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Traumatic brain injury (TBI) and spinal cord injury (SCI) are devastating conditions leading to major disability and a substantial socio-economic burden. Currently there are no effective treatments for these conditions. The development of clinically meaningful biomarkers for CNS traumatic injury remains a major clinical strategy. Imaging the neuroinflammatory (NI) response triggered by CNS injury is evolving as a key diagnostic and disease monitoring approach. The most established biomarker for the in vivo imaging of NI is the translocator protein 18 kDa (TSPO), whose expression is upregulated in response to CNS injury. We investigated the use of TSPO selective PET and SPECT ligands to image the NI response in two experimental neurotrauma models. A controlled cortical impact was used to induce traumatic brain injury (TBI) in adult mice and SPECT/CT imaging was carried out at day 7 post-injury using the [123I]-CLINDE SPECT TSPO radiotracer. A controlled contusion spinal cord injury (SCI) at thoracic T10 level was performed in adult rats (n=8) and PET/CT imaging was carried out at day 7 post-injury using the GE-180 TSPO PET radiotracer. Clinical PET imaging provides higher spatial resolution and hence better suitability for imaging SCI. Differential radioactivity uptake in vivo was detected and quantified in the injured tissue, around the primary injury site, in both injury models, compared to sham-non CNS injured animals (TBI %ID/gr: injured right hemisphere 0.022±0.001 vs. naïve right hemisphere 0.01±0.0001; SCI %ID/gr T10 spinal cord area: 0.5±0.05 SCI vs. 0.3±0.07 laminectomy vs. 0.2±0.003 naïve non-injured; P<0.001; ANOVA). Specific TSPO ligand binding was confirmed by ex vivo biodis-

tribution and autoradiography of injured tissue. Immunohistochemistry showed a high level of TSPO expression at the injury site in both SCI and TBI animals compared to that in naïve animals. This study shows the significant potential of these imaging tracers as sensitive clinical tools for non-invasive monitoring of the NI response and, potentially, of the response of NI to new therapies.

OA10W1 Seeing more matters: A short introduction to Ultrasound imaging in preclinical research with a focus on Reduction and Refinement

Meyer, Sandra, Presenting author
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Ultrahigh-frequency ultrasound (UHF; also termed Ultrasound Biomicroscopy) offers a complete and versatile imaging solution for basic and translational research in a wide variety of preclinical animal models. UHF's non invasive nature, short acquisition and anesthesia times make it an ideal tool for longitudinal studies monitoring various parameters, thereby reducing the amount of animals in a study and refining data collection. UHF is a standard imaging modality and widely accepted due to it's low running costs and non-ionizing and non-invasive characteristics. Providing excellent temporal and spatial resolution, state of the art UHF provides anatomical information with a resolution down to 30 microns in 2D, 3D and also in 4D. Further, UHF allows to display and measure blood flow and the movement of tissue and offers advanced quantification tools like Strain or contrast analysis.
On a practical level, these capabilities enable the researcher to assess systolic and diastolic cardiac function, detect orthotopic tumors and monitor tumor progression, document changes in various organs, tissues and vasculature, or follow embryonic development.
When combined with photoacoustic technology (PA) UHF can also provide molecular information based on endogenous contrast or using additional biomarkers.
Together with integrated physiologic monitoring, UHF offers reliable and reproducible research data in various research fields without compromising on animal welfare.

OA10W2 Introduction to Optical and Multimodal Imaging In Vivo: 2D and 3D Imaging of Luminescent and Fluorescent Signals in Small Animals in Combination with µCT and PET Imaging Data

Koop, Ronald, Presenting author
PerkinElmer

Conventional animal imaging requires sacrificing multiple animals at numerous time points, then slicing and staining tissue to identify the location and state of particular molecules at a point in time. However, it is much easier and more instructive to image the whole body and follow the biological processes within a live animal with no need for surgery or other invasive techniques.
In Vivo Optical Imaging has become a widely accepted technology for many different therapeutic areas. It allows researchers to follow disease progression and drug response more precisely than they can from dissecting organs.
Because you can use each animal as its own control, something that would have been lost in the noise becomes very clear. In addition, whole-body imaging means not just better data, but also new types of data. For example, disease processes often start well before symptoms become evident and using new optical imaging technologies, researchers led by Stanley Prusiner at the University of California, San Francisco, detected the onset of a mouse equivalent of neurodegenerative conditions such as Creutzfeldt–Jakob disease nearly two months before behavior was affected.
Non-invasive bioluminescence imaging of tumor cells labeled with luciferase has proven to provide invaluable information on the tumor location and burden in animal models of human cancers. Bioluminescent and fluorescent labeled tumor cells have added new dimensions for tumor detection through optical imaging. Application of optical imaging in preclinical settings has facilitated development and validation of new therapeutic agents. Recent technological advances in fluorophores and optical signal detection technology made rapid sequential imaging and quantitation of bioluminescent and fluorescent signals possible, using a single instrument. This allows collecting additional data on the biological status of the tumor, such as receptor expression, invasiveness, and apoptosis rate.
Furthermore, combining functional and tomographic optical imaging data with structural imaging modalities, such as µCT, enables deeper insight into disease processes. Integration of clinical translatable PET data gives the scientists great flexibility to analyze disease progress and mechanisms with a clear path to a relevant clinical biomarker development.
The technology allows to create cohorts of animals, or administer treatments according to how much a tumor has grown or an infection has spread. This enables the elimination of some of the animal-to animal variation, which can be a powerful way to show the efficacy of certain drug compounds and is regularly used in applications for the approval of investigational new drugs. It is possible to get a lot more data points per group of animals using these technologies.

OA10W4 Practical experimental and welfare considerations for preclinical imaging

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The use of imaging technologies is increasing in biomedical research due to their great scope for non-invasively studying biochemical and biological processes in the living animal. Their application represents a major impact on the refinement of in vivo studies in animal models, in particular for allowing longitudinal monitoring of the onset and the progression of disease within the same animal, and studying the biological effects of drug candidate and their therapeutic effectiveness. They provide a very useful set of tools for a more rapid, efficacious and cost-effective use and characterization of animal disease models, with great potential for translational research. But the use of imaging procedures can affect animal physiology, and the need to anaesthetise the animals for imaging entails potential health risks. During anaesthesia, there is an inevitable autonomic nervous system depression which induces cardiovascular depression, respiratory depression and induces hypothermia. In addition, certain imaging modalities require the use of ionizing radiation or the administration of contrast agents or imaging biomarkers, which also have consequences for animal physiology. Other procedures associated with imaging such as animal preparation (e.g. fasting, premedication), blood sampling and dosage injections can also affect physiology and animal welfare. All these factors are likely to have confounding effect on the outcome of the imaging studies and pose important concerns regarding the animal's well-being, particularly when imaging immune deprived animals or diseased animals. We will discuss these challenges and considerations to maximise efficacious data while promoting animal welfare.

OA2S2 Unintended variables for Animal Welfare Bodies to consider when assessing experiments involving behavioural tests.

Anne-Marie Farmer

Recent years have seen an increasing number of projects presented to Animal Welfare Bodies which incorporate the use of an array of behavioural tests, many of which are apparently easy to perform. Such tests are sometimes presented by scientists as providing "environmental enrichment" for the rodents involved because they have low harm impact. Nevertheless, there are aspects of this type of work that require careful consideration to ensure that the robust scientific data is generated and that their interpretation is valid. Such factors might be considered by AWBs, not least because they can impact on the animals' behaviour. By taking selected examples, this talk will illustrate how apparently simple tests that evaluate motor function can be influenced by environmental factors and experimental context.

OA9M1 Preclinical and Translational Imaging

Dr Sally-Ann Emmas, Presenting author
AstraZeneca

An overview of imaging modalities and techniques and how these can be used in preclinical research across disease areas. This presentation will focus on translational imaging and how preclinical imaging data can be used to guide clinical trials. Practical considerations will be highlighted and how this can influence the choice of imaging technique selected. In addition, impact upon the 3Rs will be discussed.

OA10W3 Preclinical MRI

Dr Martin Ilg, Presenting author
Bruker

When compared to its older cousins chemical NMR and clinical MRI, aMRI is a small but growing field. Its "patients" are laboratory animals of all kind with rats and mice dominating. Its operational use is in drug- and basic biomedical research. aMRI scanners today are smaller version of human MRI systems employing the same features for fast and easy operation.
MRI is the modality which undisputedly gives the highest combination of soft-tissue anatomical and functional information, is perfectly safe and has therefore become the premier clinical imaging technology. All its advantages apply also to living mammals and as such it plays an important role in molecular imaging.
In order to keep the relative geometrical resolution between mice and man, the absolute resolution has to be increased tenfold in each dimension - down to 30micron today. This requires extreme high field magnets which peak out at 18T and cryo-cooled RF-coil technology.
In the future aMRI will increasingly work together with other imaging modalities for small rodents: scanners for simultaneous MRI/PET - acquisition are under development.

OB1S1 Is biocontainment incompatible with animal welfare?

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In animal studies with infectious microbiological agents, it is necessary to use biocontainment methods in order to protect personnel and environment from spread of zoonotic diseases.
When biosafety level (BSL) 3 or 4 is used, it is often regarded as difficult or impossible to give the animals an environment similar to that used for uninfected animals, particularly regarding large animals such as non-human primates (NHPs). But does it really have to be so? The presentation will give examples of measures to increase the animal welfare without compromising the biosafety, e.g. the use of bedding to increase foraging time in BSL-3 cages for NHPs, a schedule for varied environmental enrichment, pair housing, and positive reinforcement training. At the same time, the proper use of personal protective equipment and Standard Operating Procedures for cleaning and decontamination of bedding, cages and equipment are prerequisites for maintenance of the biosafety and must not be compromised.

OB1S2 Comparative review of animal welfare management and refinement implementation: constraints in different levels of containments (including BSL-3) between rodents, carnivores and agricultural species in different contexts of use (human and animal health, R&D and QC testing)

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The implementation of animal welfare and procedure refinement includes: housing, enrichment, exercise, procedure methods, socialization, handling and clinical follow-up of the animals. For all animal species housed in BSL3 facility, especially when the pathogen can be transmitted by air, the handling procedure must be adapted to minimize the risk of transmission to staff, and at the same time, to maintain the highest degree of animal welfare. A right balance between these two aspects is critical.
A risk assessment is conducted for each combination of pathogen and type of use, allowing including animal care and welfare issues in the analysis as well as the need to use or not a primary biocontainment, to define which type of biosafety cabinet and / or special protections are required. For rodent and avian species, the use of isolated ventilated cages or biosafety cabinets, strictly procedured handling practices and validated frequencies of animal inspection and care are some of the very important points to address. Adapted types of enrichment are also a key parameter to refine animal procedures in BSL3 environment. Both ethical and technical parameters are taken into account with primary bio-containment. For animal health studies, our facilities designed for non-rodent species such as pets, cats, avian species, pigs, cattle, have been renovated to allow group-housing on the ground (full surface) except in isolators for avian species. Specific enrichment (platforms, perches, baskets, chains, scrapers ...) are used according to species and housing conditions and can be decontaminated or discarded at the end of the study. Dogs are allowed to performed exercise in a dedicated room or in a corridor. Procedure rooms can be located into each small biocontainment unit or can be located separately into the building with an access by a corridor. For avian species, pigs and cattle, the procedure room is used only for euthanasia and necropsy, however animals can be isolated in a corridor into the housing room. Specific equipment has been implemented for cattle in order to refine handling and realization of procedures. A working group is currently working on socialization improvement for dogs, cattle and pigs. For animal clinical follow up, a video surveillance is implemented in several rooms (cattle and pigs) and a second clinical follow-up can be performed during the afternoon. For large animals (horses) used in GMO containment, the main constraints as compared to rodents are exercise and effluents reduction. The horses are housed in small groups in large boxes. Associated to specific enrichment (brushes, toys) this contributes to maintain a minimum level of activity. The ground is covered with rubber and use of bedding is limited to long studies. A handling corridor specifically adapted to horses is included in the corridor of the facility.

OB2S1 Non-human Primates in Research

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The EU Directive 2010/63 states that given the present state of scientific knowledge the use of non-human primates in biomedical research is still necessary. However, the complex social behaviour, needs and skills as well as ethical issues around the use of these highly developed animals requires specific attention. The use of NHP in biomedical research and the dilemma's that can arise between research questions and the natural requirements of NHP will be discussed.
Well-defined animal models are often used to study fundamental properties of diseases and biological processes that cannot be studied in in vitro or in silico models. For selected biomedical research questions, non-human primates (NHP) provide the most suited animal model. Because humans and NHP share many physiological, behavioural and genetic characteristics and susceptibility to various diseases, NHP contribute to our understanding of biological phenomena and disease processes and they are important in the development of new therapies, drugs and vaccines. However, the use of NHP also raises specific ethical and practical issues. The majority of non-human primates used in Europe are macaques and common marmosets. Because these animals are susceptible to many infectious diseases that also affect humans, they provide valid models to study new therapies and vaccines. In the past, these animals have been instrumental in the development of vaccines for infectious diseases like polio and yellow fever. Currently, NHP are used to study important (re)emerging viruses, such as Dengue, Chikungunya and Zika. In addition, NHP are important in the biomedical research on non-viral infectious diseases, such as tuberculosis and malaria. These studies are usually used to analyse immune responses and protective capacity of new vaccines or drugs, but they also provide controlled conditions to unravel complex interactions between pathogens and their host. Besides infectious diseases, non-human primates are also used to study non-infectious diseases that affect humans. Examples are immune-related disorders such as MS (EAE models) and neurodegenerative diseases, such as Parkinson's disease. These NHP models are providing insight on immunological mechanisms and are instrumental in the development of new therapies. NHP are also often used to enhance our knowledge on the functioning of the brain. Studies using NHP in this type of brain research not only provides more scientific knowledge but results also in the development of new devices for disabled patients, e.g. to move again. Non-human primates have been and are still essential in the advancement of biomedical research, but we also have to be aware that this has to be accompanied by the most optimal care we can give them.

OB2S2 Vaccine Development Using Non-Human Primate Models: Bridging One Health and Biomedical Science Through a Translational Research Program

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This scientific session will introduce the Vaccine Research Center (VRC) at the National Institutes of Health (NIH) and the use of non-human primate models for human vaccine development such as Ebola, Dengue, Zika, HIV, Influenza, SARS, MERS, tuberculosis and malaria. The seminar will also evoke newly developed and existing NHP vaccine models and biotechniques, biosafety challenges (ABSL3 and ABSL4 veterinary practices), strategies for refinement, and One Health implications.
The main objective of the VRC is to establish mechanisms of inducing long-lasting protective immunity against a variety of pathogens that present special challenges to vaccine development. The Translational Research Program (TRP) at the VRC provides all aspects of oversight and programmatic assistance to support teaching, training, and in vivo research by managing all preclinical safety and regulatory issues, ensuring judicious and humane use of animals in compliance with all institutional, local, state, and federal guidelines. The TRP also pursues independent and collaborative research projects related to animal model and preclinical product development for HIV, influenza, tuberculosis, emerging infectious diseases such as alphaviruses, and other biodefense-focused diseases. Pathogens of Interest at the VRC The scientific program at the VRC has included HIV, Influenza, Dengue, Ebola, tuberculosis and malaria. Most recently Ebola and Zika viruses have caused significant outbreaks and provide excellent examples to illustrate how directives from the federal government initiate rapid response at the scientific level here at the NIH. An expert team at the VRC is rapidly organized and together create non-human primate models of vaccine delivery and viral challenge, while always adhering to the ethics, regulations and policies set forth by the VRC ACUC, and NIH as a whole. Even with the urgency with which studies are proposed and designed, corners are never cut and a rigorous program of review is always conducted with the highest level of animal welfare at the forefront. Solving diverse challenges at the VRC Many challenges faced and solved include pair and group housing of experimental animals, behavior training for repeated biosampling in conscious animals, adoption of novel infusion techniques to eliminate repeated inoculations, establishing a multi-site electronic medical records system for effective communication and adoption of standard clinical endpoint criteria for effective veterinary case management. There is an active program for refinement of an array of veterinary biotechniques. Lastly, the significant challenges involving ABSL3 and ABSL4 studies cannot be underestimated. Training programs in biocontainment and concurrent animal use exist nationally that have demanding requirements for personnel wishing to be employed in these special use animal facilities.

OB2S3 The need for better justification of research using non-human primates

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The European Directive imposes conditions on the use of animals, especially non-human primates (NHPs) that require demonstration of benefits and application of the 3Rs. The refusal of many organisations to acknowledge the limitations of research on NHPs for advancing human health and the failure of investigators to fully apply the 3Rs, damages the case for using NHPs, polarises the debate on animal use, and undermines efforts to improve animal research and develop alternatives.
The European Directive (1) recognises that the use of non-human primates (NHPs) in research may be necessary, but only on condition that the research leads to medical, veterinary, scientific or educational benefits, and that the 3Rs are applied. While these stipulations apply to all animal research in the EU, few would disagree that adherence to the law is even more important when NHPs are being used. Unfortunately, the need for greater justification has led to exaggerated claims for the importance of NHP research and the extent to which the 3Rs are applied, which do not stand up to scrutiny. A number of examples will be given. On the importance of NHP research, the claims are particularly strident for advancing human medicine, presumably on the grounds that this is more important than the other categories of justification (veterinary, science and education) and will carry more weight with regulators and funders. While many individual scientists are willing to acknowledge the limitations of animal work in predicting what happens in man, several organisations representing scientists, or involved in funding and regulation of research, seem obligated to defend NHP work even when it is demonstrably not essential. On the 3Rs, these same organisations make sweeping generalisations about the exemplary standards of European science, while turning a blind eye to the real evidence of poor welfare, inadequate attention to alternatives, and the low quality and lack of reproducibility of much animal work. In doing so, these organisations weaken rather than strengthen the case for NHP research. Their statements serve to polarise the debate, present scientists as pro-animal extremists, and provide an easy and legitimate target for those opposed in principle to animal work. By defending the indefensible, they undermine genuine efforts to promote better quality animal work and the development of alternatives. Scientists, funders and regulators must do better. They should not hide behind regulations that are not followed in practice. Justification must acknowledge the scientific limitations of animal research. Ethical decisions must be based on evaluation of realistic benefits versus actual harms. Finally, they should remember that it is society at large, not science and scientists, that defines the line between acceptable and unacceptable use of animals. * The views put forward in this paper are those of the author, not NC3Rs policy

OB2S4 Validation of a Multiplex Antibody Diagnostic Test for Tuberculosis in Nonhuman Primates

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Tuberculosis (TB) in nonhuman primates (NHP) is highly contagious and often produces rapid disease thus making it important to have a timely detection of an outbreak. Current tests including TST used for routine NHP colony screening lack desirable sensitivity, specificity, efficiency and/or throughput. A new blood based multiplex immunoassay was developed to detect antibodies in Mycobacterium tuberculosis (M. tb.) infected NHP's that can be used potentially for routine colony surveillance.
Material and methods: We aimed to develop an immunoassay for plasma antibodies against M. tb. A key challenge was that not all infected animals contain antibodies against the same M. tb. antigen. In continuation of our previously published proof-of-concept studies we used a Luminex bead based multiplex panel with 28 M. tb antigens/assays in the current validation study. Antibody levels were examined in plasma samples from several cohorts of well characterized specific pathogen-free (SPF) colonies at several facilities including the Charles River Laboratories. A total of 684 healthy macaques were used in the validation study to determine specificity of the test. Sera from two different rhesus macaque (Macaca mulatta) colonies (n=460) and two cynomolgus macaque (Macacca fasicularis) colonies (n=224) were used in the study. Sensitivity of multiplex bead panel was assessed by testing sequentially collected sera from experimentally infected rhesus with M. tb. strains (Erdman, n=6 and H37RV,

n=4) and cynomolgus macaques (Erdman, n=9) at various time points post infection (8, 12, 16 and 24 weeks). Results: The cut-offs were calculated using data from SPF NHP's and overall specificity of the test was found to be >95%. Data revealed antibodies against seven antigens/assays including ESAT-6, CFP-10, ESAT-6/CFP10 fusion, PstS1/P38, HspX, Ag85b and C-E fusion proteins are the most consistent with infection. Antibodies against both M. tb. Strains (Erdman and H37Rv) appear as early as 8 weeks in experimentally infected rhesus and cynomolgus macaques. A similar antibody pattern was observed in naturally acquired M. tb. infection in rhesus macaques (n=15) with positive lung TB pathology (n=10). The panel sensitivity was between 80-100% at various time points (8, 12, 16 and 24 weeks) during seroconversion. Conclusions: Multiplex immunoassay was validated for use in the routine detection of TB in NHP's. Findings from this study suggest that antibody profiles may vary with progression of M. tb infection in NHP's, presumably because antibodies reflect changes in antigen expression during the course of infection. High sensitivity and specificity of our test strongly suggest that this user-friendly and easily implementable multiplex bead panel, containing 7 M. tb antigens, may provide a high-throughput alternative for TB screening.

OB3S1 Non-human primate aerosol challenge models of tuberculosis: Advanced in-life imaging to refine and improve efficacy studies for TB vaccines and therapies

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Dennis MJ, Taylor I, Gleeson F, et al, Co-Authors

CT scanning of macaques infected with TB by aerosol has been established by using a high containment pod. Disease has been identified early after infection before the onset of clinical signs. This presentation will show the results of these early infection, low challenge dose studies and will discuss the ethical implications in terms of refinement and reduction that will result from the ability to detect early stage disease following the administration of realistic low doses of the pathogen
A third of the world's population is estimated to be latently infected by TB. The devastating effects have been exacerbated by emergence of resistant strains and HIV. There is an urgent need for vaccines. NHP represent the most relevant system to predict safety, immunogenicity and protective efficacy of vaccines prior to their introduction in humans. Humans develop tuberculosis following inhalation of airborne particles; low-dose aerosol infection of NHP represents a close approximation to human disease. We have established aerosol challenge models of TB in NHP for the assessment of TB vaccines. The readouts currently used to measure the efficacy of new TB vaccines in NHP models have limited sensitivity, requiring the use of high dose challenge with the resultant risk that vaccines with the potential to be efficacious against natural challenge could be disregarded. Therefore, there is a need to refine the existing NHP models and define a low-dose challenge system. 'In-life' imaging using computed tomography (CT) allows disease development to be quantified over time from early stages of infection. Within our early studies, we have harnessed the power offered by the modern medical imaging technique of magnetic resonance (MR) imaging to visualise pulmonary disease and assessed the potential of stereology as a tool for the quantification of tuberculosis-induced pulmonary disease following aerosol infection. Following successful in-life imaging and proof of principle studies using MRI and CT scanning, the capability to collect CT scans of macaques infected with TB has been established using a high containment pod to avoid contamination. These scans have been successful in identifying very early stages of disease before the onset of any clinical signs or detection by conventional X-radiography, and have facilitated the establishment of an ultra-low dose M. tuberculosis aerosol challenge model in macaques. Use of in-life scanning will allow assessment of vaccine efficacy without the need for animals to progress to the later stages of disease. The ability to monitor disease progression in individual animals removes the need for serial sacrifice and will consequently reduce the numbers required for studies. It is anticipated that the use of this technology will allow refinement and reduction in any infectious disease model that requires the use of NHP.

OB3S2 Influenza and other emerging viruses: benefits and limits of animal models

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Some animal viruses can cross the species barrier and infect humans. Such zoonoses can result in subclinical or mild infections in humans, but can also result in serious or sometimes even fatal disease. Because zoonotic viruses in general lack the ability of sustained human-to-human transmission, they usually do not represent threats to the public at large. On rare occasions, however, viruses may acquire the ability of sustained human-to-human transmission with the potential to cause a pandemic.
Influenza A viruses belong to the family Orthomyxoviridae and are circulating in wild migratory birds of aquatic habitats around the world. They occasionally spill over from the avian 'virus reservoir' into other animals, including poultry, pigs, horses, a variety of carnivores and marine mammals. Sporadically, the viruses adapt to the new hosts, leading to enzootic virus circulation for years, decades or centuries. Zoonotic influenza A virus infections occur relatively frequently, but mostly without serious consequences for public health in general. However, the introduction of 'new' influenza viruses from animals to humans can result in pandemics, i.e. global epidemics caused by a new subtype of influenza viruses to which population immunity is low or inexistent, as it was the case four times in the last 100 years alone. Coronaviruses infect and cause disease in a wide variety of species, including bats, birds, cats, dogs, pigs, mice, horses, whales and camels. Four coronaviruses cause annual epidemics in humans, and are associated with mostly mild respiratory illnesses: HCoV-229E, HCoV-OC43, HCoV-HKU1 and HCoV-NL63. The outbreak of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2003 reminded us that coronaviruses also can be virulent in humans, causing approximately 800 deaths among 8000 human cases of SARS detected in 30 countries. Since 2012 we have experienced outbreaks caused by the distantly related Middle East Respiratory Syndrome coronavirus (MERS-CoV). This virus caused >1600 human cases of infection, including ~600 deaths. In response to outbreaks like those caused by influenza and coronaviruses, virology laboratories frequently need to develop animal models. Animal models are crucial to develop and evaluate intervention options such as vaccines and antiviral drugs. In addition, animal model systems are needed to advance our understanding of the pathogenesis of these virus infections and to investigate modes of virus transmission. These animal models may vary substantially for different purposes. In this presentation, various animal models to study respiratory viruses during outbreaks and fundamental research projects will be discussed with a focus on influenza A viruses and SARS and MERS coronavirus. Specifically, the reason for using different animal models for different research questions will be addressed.

OB3S3 A bovine natural-host infection model enables the evaluation of intervention strategies against Respiratory Syncytial Virus for the combat of lower respiratory tract infections in kids and calves

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Respiratory syncytial virus (RSV) is one the main causative agents of lower respiratory tract (LRT) infections world-wide. The virus has a human (RSV) and bovine (bRSV) variant which are closely related and share many aspects of pathogenesis and clinical outcome. Severe life-threatening LRT disease is mostly seen in young individuals. Despite significant research activities on prevention strategies, there is still no licensed RSV vaccine. Veterinary bRSV vaccines are subject of improvement.
The bRSV infection model in calves, originally developed for veterinary vaccine testing (1), is a natural-host model which closely resembles the human situation with regard to the clinical course and severity of the infection. These features distinguish this homologue model from alternative non-homologue and semi-permissive RSV infection models in small rodents and enable the pre-clinical evaluation of efficacy and safety of human RSV vaccine candidates and therapeutic interventions. Besides, the calf model can be used for more fundamental or proof of concept studies within biomedical research (2). Young calves are experimentally infected by aerosol with 103 to 104 log10 TCID50 of an in-vivo passaged bRSV field strain. After inoculation, calves are closely monitored over time including daily clinical observations and specific assessment of respiratory symptoms. The model offers the possibility for repeated sample collections (including nasopharyngeal swab, lung lavage and blood samples) to assess virus replication in the upper and lower respiratory tract and various immune responses. At the end of study, necropsy is performed for (immuno)histopathological examination of the lungs. Upon inoculation, calves develop moderate to severe respiratory symptoms including nasal discharge, coughing and dyspnea usually in combination with fever and depression. Clinical symptoms and virus titers peak between day 5 to 9 after inoculation. Lung pathology typically reveals macroscopic lesions with histological changes of bronchiolitis or pneumonia. Primary readouts in evaluating efficacy and safety of intervention strategies are a reduction of clinical disease, virus replication and lung pathology. Besides, innate and adaptive immune responses can be studied for secondary readouts like correlates of protection or safety and for a better understanding of the pathogenesis and host-pathogen interactions (3).The bovine RSV model also allows the use of more designated clinical and physiological approaches like telemetric devices to measure body temperature and respiratory rates, collection of arterial blood for monitoring blood gasses and bronchoscopy sessions including collection of lung biopsies. In conclusion, the bovine RSV model is a homologous, predictive animal model and clearly displays a clinical disease very similar to human RSV infection in infants. This allows the model to be used in veterinary research and represents a valuable pre-clinical model in human research.

OB3S4 Safe science? Contemporary risks for humans and animal health - Taking risks with today's guidelines

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Idexx Bioresearch

Current screening guidelines are in place for certain laboratory species. The aim is to standardise surveillance towards pathogens for animals and for humans. However some laboratory species are not yet taken in consideration, some risks probably over estimated and some other under estimated as, for example the risks derived by the use of biological materials. This talk wants to critically review existing recommendations and to outline data on existing risks that are not yet considered by them.
Disease surveillance and health monitoring are very important to standardise research, improve well-being, minimise spreading of diseases and to certify quality and assure there are no risks for human health when working with laboratory species. However, at the moment, there are guidelines and recommendation only for the main laboratory animal species and not all is known by professional staff about other species. In many facilities there are procedures in place to conduct health monitoring on mice, but what about other species, such as zebrafishes, other rodent species? How many facilities are routinely testing biological materials? Do we really know the risks for animals and human health we are exposed to when testing only according to FELASA guidelines? Can we introduce unknown disease dealing with patient derived xenografts or with other biological materials? This talk want to critically review current guidelines, to look at few risks for human health dealing with other species, to overview incidence data of contamination of biologicals samples by rodent and human pathogens and also to present a case study that emphasize the fact that we all need to be prepared to possible challenges.

OB4M1 Over nutrition: a societal issue that affects our animals

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Over nutrition is a societal challenge affecting humans and their pets. Obesity is caused by an imbalance between energy intake and energy expenditure resulting in a net positive energy balance. Reasons for this imbalance are complex, including socio-economical, genetic and life-style factors. By studying mechanisms leading to overweight across species, precious knowledge can be obtained within two central fields of the global One-health framework; Comparative medicine and the human-animal bond.
Studying spontaneously arising pet obesity holds great potential for understanding the human counterpart. Distinct hormonal alterations may be similar or different to observations in humans, one example being circulating leptin levels that increase in correlation with increasing fat tissue in all species. On the other hand, Adiponectin, a "good" adipokine believed to counteract insulin resistance decreases significantly in human and rodent models while in dogs, circulating levels seem to exceed human levels and the suppression by obesity is less severe. Insulin resistance develops in pets in response to obesity – however it seems the canine β-cells can compensate for the increasing demand while feline β-cells deteriorate predisposing to diabetes. Due to intensive breeding programs, a diverse selection of breeds with specific genetic and phenotypic traits has evolved. These specific traits include differences in appetite regulation and propensity to spontaneously engage in physical activity. Studying differences between breeds help understanding the role of genes and metabolic traits. Investigating the benefits or adverse effects of human–pet interaction to human and pet health and wellbeing may lead to new multifaceted interventional strategies. Factors affecting risk of obesity in dogs has been categorized as dog specific and owner associated. The pet owner controls the type and size of the meals and treats as well as the exercise level. With this in mind it seems puzzling that obesity is an increasing problem in our pets. One could expect that the owners were aware of the detrimental effects of obesity also in their pets and wanted to prevent it from developing. In a survey, veterinary practitioners estimated that only 3% of canine obesity cases could be attributed to dog specific factors while 97% was attributed to owner-related factors. In addition to diet and exercise, owner perception and attitude is important, many owners do not perceive their pet as overweight, 25% of owners would

not act on being informed that their pet is overweight and if actions should be taken, dietary intervention would be acceptable while changes in exercise level and treats are less tolerable. Finally, to underscore the value of a One-health approach in this area, while the risk of obesity in cats is not associated with owner body mass index, obese dog owners are more likely to have obese dogs compared with normal weight owners.

OB4M2 Environmental issues: should users have concerns with Lab Animal diet?

Martel, Dominique, Presenting author
SAFE

Diets are mixed of vegetal and animal ingredients. These raw materials are mostly produced in natural environment, and their quality depends on their grow environment (industrial, urban and agricultural practices), their history (production, transformation, transport, storage...) and their National regulations.
The new knowledge, new analytical means, new assessment tools provide relevant information on potential risks on contaminants, mainly chemical. At first, an overview on the source and manner to contaminate ingredients will be developed. Afterwards, a panel of the most common and main pollutants will described, and their related risks.· With the extent of plant protection practices all over the world and for a large panel of vegetal production, a focus will be made on pesticides hazards with examples of detrimental evaluation,· The contaminant issue information will also feature Persistent Organic pollutants (POP), Mycotoxins, new Toxics,· With the climate change, the aflatoxins could be a new emerging risk on cereals produced in UE. An investigation and prediction about this increasing risk on Maize and Wheat will be described. Finally, the tools to master, monitor and ensure a low environmental chemical risk will be implemented: regulation, alert network, analyses, commitments of the food chain suppliers, specific ingredients and formula design. These gathered different tools must ensure the safety of lab animal diets for a safety research. As for food chain (from Field to Fork), Researchers and Lab facilities manager are spurred to take care from Field to caging hopper according to their research purpose.

OB4M3 Making Replacing Primate Experiments Possible: Unconventional Recommendations of how to Progress

Hudson-Shore, Michelle, Presenting author
Fund for the Replacement of Animals in Medical Experiments (FRAME)

Primate use provokes passionate and opposing exchanges. Such disagreement has created an impasse preventing the exploration of if and how primate use could be ended. Limited investigation of the animal experimentation debate has so far brought no understanding of the extreme polarisation of the issue. This PhD project expands knowledge in this area exploring it from a novel perspective using a multi-method approach to examine the primate debate and uniquely consider how to overcome the deadlock.
Part of a broader PhD thesis is reported here. Two contrasting case studies were conducted, involving semi-structured interviews with primate and non-primate users in schistosomiasis and Parkinson's disease research. Analysis of the responses revealed some interesting areas of consensus and important differences in the justifications given by different users, and between the two fields of research. The key finding is the recognition of the fundamental role that social dynamics of science play in explaining why the primate impasse persists. Social themes and theoretical perspectives can be applied to understand how and explain why science is conducted in the manner that it is. Exploring the interviews through particular dynamics — such as competition and reputation, expectations, core sets and publications, entrenchment and policy, and ethics and speciesism — indicates that, in certain circumstances, the impasse can be overcome. This presentation gives some constructive recommendations on how progress might be achieved, focussing on those which require improved collaboration and communication. Implementing these recommendations will involve various stakeholders and will have important consequences for primate and alternative research practices, and science communication.

OB5M1 Challenges and opportunities in therapy of human epilepsy

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Epilepsy is one of the most prevalent neurological diseases affecting approximately 1% of human population. It is characterized by occurrence of seizures due to abnormal excessive and synchronous neuronal activity in the brain. Epilepsy is associated with seizures and a variety of serious and life-threatening comorbidities. This chronic and debilitating disease impacts not only on the individual patient, but also on the family and indirectly on the entire society.
Antiepileptic drugs (AEDs) are the mainstay of therapy in epilepsy, but approximately one third of patients still suffer from uncontrolled seizures. These drugs are also not able to prevent or change the course of the disease and are not effective against the comorbidities associated with epilepsy. Consequently, there is an urgent need to develop novel, more efficacious therapies that would be able to modify the underlying pathology of the disease and alleviate its comorbidities. This significant challenge requires innovative approaches to the discovery and development of such innovative therapies. Recent years have brought significant discoveries in the field of epilepsy, which created great opportunities for introduction of novel therapies or diagnostic tools. For example, next generation sequencing led to the discovery of several genes linked with different forms of epilepsy. Together with advanced brain imaging approaches these technologies have fundamentally changed the therapy paradigm in some epilepsy syndromes enabling precision medicine and personalized treatment choices for patients. There have also been important advances in experimental epilepsy research. Novel disease models are being developed and more routinely used to validate innovative targets for AEDs and to provide proof of concept support for future clinical development. We now not only have rodent models closely recapitulating human disease, but model systems such as zebrafish, drosophila or worms are increasingly used in preclinical research. Furthermore, induced pluripotent stem cells, which can be derived directly from patients' tissues (e.g. skin) and differentiated into neurons, are an invaluable resource in epilepsy research. Collectively, these state-of-the-art discovery tools allow probing mechanisms that had not been previously considered as targets for AEDs. For example, emerging data indicate significant importance of non-neuronal cells, like microglia and astrocytes, in the pathophysiology of epilepsy. Significant advances in our understanding of epilepsy pathophysiology have been made over the recent years creating unparalleled potential for future therapeutic opportunities. The challenge in translating these exciting discoveries into new treatment options for patients still remains, but may be leveraged with the use of emerging technologies and novel disease models closely linked with human disease.

OB5M2 Epilepsy and seizure dogs: to live again...

Thienpont, Caroline, Presenting author
Hachiko

Traditionally, working dogs have served people by assisting in a wide range of tasks, from herding livestock, over detecting explosives or narcotics, to performing in search and rescue operations. During the past two decades, however, particular attention is being paid to the effects of the human-dog bond on mental and physical human health.
In a condition of epilepsy, additional challenges exist because, the majority of patients experiences problems even with loss of consciousness. They are thus at risk for injury and, following an ictal event like a tonic-clonic seizure, they might be too disoriented to think about their medication immediately after a seizure. Consequently, this patient group particularly benefits from having a full time canine assistance companion. In 2003, Hachiko was the first non-profit organization in Belgium to start training seizure dogs. Currently, they have trained and placed 11 seizure response dogs. A seizure response dog is trained to perform one or more tasks, usually immediately following an epileptic attack. Examples of tasks include pressing an alarm button, waking up the patient, and fetching medication or the phone. The dog can also assist in the safe execution of everyday activities, such as walking down the street. Seizure response dogs are intended for people with documented epilepsy for whom medication or other treatments were either ineffective or are not implementable because of a low prediction of success. Anecdotal and scientific reports describe that some seizure response dogs as well as pet dogs, prior to the onset of an epileptic event in humans, spontaneously display typical behaviour that is interpreted as a warning signal for the oncoming ictus. It is believed that this behaviour is associated with changes in the patient that are perceived by the animal. Due to a dog's apparent sensitivity to the subtle changes within the patient, it is possible to train dogs to alert an owner that an epileptic seizure is likely to occur in the near future. This is life changing, and that is what Hachiko does. Currently there are seizure dogs in Switzerland, Russia and hopefully soon in Wallonia, France and Croatia thanks to the workshops presented by Caroline Thienpont.

OB5M3 Opportunities for improving animal welfare in rodent models of epilepsy and seizures

Lidster, Katie, Presenting author
NC3Rs
Jefferys JG, Blümcke I, Crunelli V, Flecknell P, Frenguelli BG, Gray WP, Kaminski R, Pitkänen A, Ragan I, Shah M, Simonato M, Trevelyan A, Volk H, Walker M, Yates N, Prescott MJ, Co-Authors

Animal models of epilepsy represent an important area for application of the 3Rs; with the potential for refinement of seizure induction, maintenance and monitoring to minimise any pain or distress and improve scientific outcomes. The UK National Centre for the 3Rs (NC3Rs) convened an expert working group with the aim to review the current use of rodent models of epilepsy and seizures and identify opportunities for refinement. The working group surveyed the international epilepsy community to identify which mammalian models are used in epilepsy research and to define best practice. In addition, a systematic review of the scientific literature was carried out to identify supporting evidence. The survey, literature review and expert opinion and practical experience of the members of the working group were used to define recommendations. Recommendations include induction procedures, in vivo recordings, perioperative care, welfare assessment, humane endpoints, social housing, environmental enrichment and reporting of studies and data sharing. These recommendations provide researchers, veterinarians and animal care staff with the tools to refine the use of rodent models of epilepsy and seizures. In addition, model-specific adverse effects and refinements were identified for commonly used models. Areas where increased knowledge and technological development would facilitate refinement and best practice were identified. Opportunities for refinement of the use of rodent models of epilepsy and seizures have been identified; implementation of the recommendations could help to improve the quality of animal studies in epilepsy research and maximise the use of animals.

OB5M4 Harm-benefit analysis in epilepsy models

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Epilepsy, which is the propensity to have recurrent seizures, is commonly modelled in rats and mice. Models of epilepsy and seizures may cause pain, suffering, distress and lasting harm (PSDLH). The severity of PSDLH depends on the model used and the refinements applied, but typically, models of epilepsy are categorised as moderate or severe under EU Directive 2010/63/EU.
The requirement to undertake a harm-benefit analysis during project evaluation is set out in 2010/63/EU in order to determine whether the likely harms experienced by the animals are justified by the expected benefits. Humans with epilepsy are often unaware during seizure events and it is suspected that this is also true of animals. However, suffering in epilepsy models does not necessarily occur during the seizures themselves. The seizure induction methods, secondary pathophysiological changes, comorbidities, post-ictal sequelae, instrumentation for monitoring or stimulation, husbandry practices and seizure monitoring, may also cause PSDLH. Therefore, the whole epilepsy syndrome needs to be taken into account when evaluating the harms including the duration, frequency and intensity of the adverse effects. Epilepsy models should be refined to minimise suffering whilst ensuring the scientific objectives can still be met. Typical refinements include minimising status epilepticus duration and seizure severity, optimising husbandry such as social housing, maintaining normal hydration, refining drug administration routes, use of radio-telemetry instead of tethered systems, use of the mouse and rat grimace scales, and balanced analgesia and anaesthesia protocols. The benefits considered during harm-benefit analysis are the specific, expected beneficial outcomes of the objectives of the project. The assessment of the possible benefits of an epilepsy project can be facilitated by answering the following questions: What are the expected benefits of the work? Who and how many will benefit? How will the benefits accrue? When will the benefits be achieved? The benefits of an epilepsy project are maximised and harms minimised by ensuring that the most scientifically relevant and least severe model for the scientific purpose is identified, whilst also ensuring that any model-specific refinements are applied. There is the potential for severe suffering in epilepsy and seizure models, however model-specific refinements may make the harm-benefit analysis more favourable. Since animals are unlikely to be aware during seizure events themselves, the key to refinement in epilepsy is to consider the whole epilepsy syndrome being induced, with the associated morbidities.

OB6S1 Animal models of pain: anatomy, physiology and comparative considerations

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Altered plasticity of the nociceptive system is responsible for chronic pain, and results from sensitization of the nociceptors, the primary sensory neurons involved in the processing of pain-related stimuli. An important feature of the nociceptive system is that reactions to noxious stimuli (i.e. the actual or potential tissue damaging events) can be modulated by local circuit interneurons that, in normal conditions, calibrate the system response to stimulus intensity and duration in time.
Pain and nociception are often used as synonyms. However, it is important to know that there are fundamental differences between nociception, i.e. the capability of the sensory system to encode a heterogeneous group of sensory stimuli that cause actual or potential tissue damage, and pain, i.e. the conscious perception of these stimuli. Importantly, animal models used in experimental research are often tested for nociception rather than bone fide pain, as most of the behavioural tests for "pain" actually measure withdrawal reflexes to avoid tissue damage, or the sensitization of nociceptors. In the clinics, chronic pain comprises several types of long-lasting pain, including inflammatory pain (e.g. arthritis), cancer pain, and neuropathic pain that follows to injury (e.g. an ischemic insult or a trauma) of the peripheral and/or central nervous system. Whereas the protective role of nociceptive pain appears obvious, inflammatory and neuropathic pain persisting after their causative event(s) is (are) ceased are a maladaptive response of the nociceptive system, and must be regarded as fully pathologic conditions. Hyperalgesia and allodynia typically characterize all types of chronic pain, whereas burning, paraesthesia and dysesthesia are additionally present in neuropathic pain. Nociceptive pathways are polyneuronal chains that originate from a nociceptor in the peripheral nervous system. Nociceptive somatic inputs from all the parts of the body except the head are transferred to second-order spinothalamic projection neurons in the dorsal horn of the spinal cord. These neurons give rise to the spinothalamic tracts reaching third-order neurons in thalamus. Nociceptive somatic inputs from the head reach the thalamus via the trigeminal system. Nociceptive visceral afferents mainly derive from the sensory ganglia associated to the vagus and glossopharyngeal nerves, are relayed to the nucleus tractus solitarii and parabrachial nucleus, and eventually reach the thalamus. From the thalamus, the nociceptive input transferred to the sensory cortex where it gives rise to the sensation of pain. Animal models of pain are described, as well as their severity and translational relevance. Models fail in two categories to, respectively, induce inflammatory and chronic pain. In the first, pain is elicited by local injection of pro-inflammatory molecules. The latter comprise spinal nerve ligation, chronic constriction injury or partial ligation of the sciatic nerve.

OB6S2 Scrapie prions: etiological agents of sporadic Creutzfeldt-Jakob disease in humans?

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Transmissible spongiform encephalopathies (TSEs), or prion diseases, are neurodegenerative disorders that affect a spectrum of mammalian species. Following the epizootic of bovine spongiform encephalopathy (BSE) in the 1980's and the emergence of variant Creutzfeltd-Jakob disease (vCJD) in humans, the in-depth assessment of the zoonotic potential of the TSE agents circulating in animal populations, including those causing scrapie in sheep, has become a major public health challenge.
The conventional method to identify an animal TSE as a zoonotic disease combines data from in vivo experimental transmission to animal models and epidemiological studies. This method can be applied to scrapie. Some rodent-adapted scrapie isolates were reported to propagate in non-human primates. However, because prions adaptation in rodents can profoundly alter their biological properties and no transmission was observed in chimpanzee inoculated with sheep scrapie, the significance of these results was considered low. Recently, mice genetically engineered to over-express the human prion protein (tgHu) have emerged as highly relevant models for gauging prions capacity to transmit in human. These models can propagate human prions without apparent transmission barrier and they were largely used to confirm the zoonotic ability of BSE. We showed that a panel of sheep scrapie prions transmitted in several tgHu mice models with comparable efficacy than cattle BSE. Surprisingly, the serial transmission of different scrapie isolates in these mice led to the propagation of prions that were phenotypically identical to those causing sporadic Creutzfeldt-Jakob disease (sCJD) in humans. Although these experimental results show that scrapie prions have an intrinsic zoonotic potential and suggest a causative link between scrapie and sCJD, they need to be qualified. Indeed, bioassays in TgHu mice cannot reproduce exactly field conditions and many epidemiological factors, such as prevalence of scrapie and sCJD, or real human exposure to scrapie strains, should be combined to assess the public health risk related to this animal disease. Interestingly, scrapie has been endemic in most small ruminants populations used for food production for centuries whereas sCJD remains a rare disease. Consequently, even if it cannot be excluded that a small proportion of sCJD cases are caused by scrapie prions, it would be an overstatement to consider scrapie as a major threat for public health.

OB6S3 The Horse as Model for Osteoarthritis Research – Research Tools vs. Stakeholders

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Today, a priori small animal models are used in osteoarthritis (OA) basic research, whereas large animal models are used for validation purpose as well as for improvements of surgical or therapeutic interventions. However, the applicability to human OA, especially with regard to the small animal models is questionable. The horse as a patient of considerable economic value also offers the opportunity to serve as a naturally occurring model of OA which parallels the human disease in many aspects.
In Germany approximately 33.7% of treated horses in the clinics suffer from diseases affecting the musculoskeletal-system and are incident to high costs. In North America the costs reach the total of $1 billion annually and the incidence for lameness lies between 8.5 up to 13.7%. Especially racing thoroughbreds are susceptible to fetlock pain and lameness (25%) leading to the early retirement of equine athletes and a high economic loss to the equine industry. Therefore, up to 60% lameness incidents are caused by osteoarthritic disorders demonstrating an undeniable need for OA research not only in men but also in horse. Moreover, when comparing the cartilage thickness among different species, the stifle joint of horses provides the closest approximation to the thickness of human articular cartilage of the knee. This comparability in size allows the applicability of comparative studies such as the examination of postoperative parameters immediately after surgery using specific scores. In addition, the horse provides the opportunity for arthroscopic interventions to obtain synovial fluid, to assess the cartilage shape macroscopically and to harvest small pieces of cartilage for molecular biological investigations. Of note, one horse provides enough material to address most research purposes

whereas lots of small animals would be needed to end up with enough material. Economic efficiency could be reached by cooperation between researchers and equine industry. In our own work, we use the horse as in vivo as well as in vitro model to effectively reduce the number of needed animals and to gain maximum knowledge for the human system. In vivo, we developed a model based on the creation of full-thickness defects with an average diameter size of 1 cm in the femoropatellar joint. The defects are filled directly after the creation. The specific protocol depends on the research question or therapeutic approach. Furthermore, the availability of tissues and cells from equine donors is much more easily compared to human resources. Therefore, we developed in vitro models for OA and bone healing based on equine cells. The gained knowledge can be used for the transformation to human system as well as a replacement strategy for the small animal model in OA research. In conclusion, as an OA patient, the horse is a suitable model for OA research. Considering animals as beneficiary and not only as research tools could give a major impact on translational medicine in the future.

OB7S1 Challenges of Working with Large Animals in a High-Containment Facility

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Large farm animals, such as swine, cattle, goats, sheep, etc. can hardly be put into isolators, but need to be kept in animal rooms or stables. Thus, containing large animals and the infectious agents with which they are experimentally infected invariably poses new and different challenges with regards to biosafety compared to the use of small laboratory animals. The animal room forms the primary barrier of containment, thus protecting the immediate surroundings as well as the environment.
Additionally, working with the animals inside this primary barrier requires additional safety measures for the animal handlers. The IVI is the Swiss reference laboratory for the diagnosis, surveillance and control of highly infectious animal diseases such as fowl pest (avian influenza), foot and mouth disease and classical swine fever. The IVI investigates the emergence of new diseases in animals and their potential for transmission to humans (e.g. SARS, MERS, JEV). It is the approval authority for vaccines and sera for animals. The IVI is part of the Federal Food Safety and Veterinary Office (FSVO).As part of its mandate the IVI is interacting with many different national and international bodies and organisations. Teaching and training in virology, immunology and biosafety form an integral part of this. The IVI is the only Swiss facility in which work with highly infectious animal diseases as well as zoonosis including studies with large animals can be performed. The laboratories as well as the animal units meet the standards of a BSL3 and BSL4 facility with regards to the protection of the environment (BSL3 Ag) according to Swiss legislation (Containment Ordinance). It has been operational since 1993 and is currently undergoing a large refurbishment and engineering update. In this presentation we would like to highlight some of the architectural features of our high-containment large animal facility taking into account our experiences over the last 25 years touching on such subjects as:
Design features and lessons learnt
Animal welfare vs. biosafety
Effluent treatment systems
Work with zoonotic agents
Fumigation (switch from formaldehyde to VHP)

Furthermore, we would like to highlight some of the challenges faced when renovating such a facility while being fully operational.

OB7S2 Practical and Operating Issues of Autoclaves associated with High Containment Facilities

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The practical and operational issues, including drafting the user specifications for animal facility autoclaves are essential consideration before being submitted to engineering or to the supplier.
An autoclave is a regular feature of animal and containment facilities to both provide sterile input and to treat waste exiting. Autoclaves are relatively simple in design and operation, requiring elevated temperature for a define time achieve by means of saturated steam and removal of air. Typically they are supplied in standard forms, chamber size, operating parameters and standardised installation. Yet the requirements for SPF, barrier and high containment go beyond the "standard" specification. The establishment and maintenance of a barrier for biocontainment at biosafety levels 3 and 4(BSL-3/4) animal facilities, with their particular load types, yet have workflows that ensure separation of space. The requirement to be able to effectively decontaminate large items and dense solid such as carcasses are particular challenges that need particular attention. There are many potential problems and pitfalls to be avoided by appropriate specification of performance, selection of good options for achieving the containment and assurances needed in animal facilities. The areas that can be problematic are; quality of steam supply; achieving through the wall barrier and maintaining it; provision or not of weirs; suitability of air filtration and alternatives. Further the user needs around sizing, location, throughput and orientation especially to assure optimised workflows and practical considerations. A good specification will also take account of ergonomics, and design of loading devices. A successful outcome will be achieve if performance is pre-planned into the design and will ensure easier sustained performance and inform support, validation, and maintenance needs. This will be of interest to those planning facilities, maintaining and purchasing autoclaves and designing Biosafety level 3 and 4 facilities to assist in avoiding unexpected expenditure and delays.

OB7S3 Risk analysis, selection, validation and safe operation of innovative biocontainment approaches: the combined use of purpose-designed IVC biocontainment units and biosafety cabinets for rodent-based research

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Allentown

Innovative IVC biocontainment equipment and related practices are expected to meet the specific needs of animal-based research, project management and facility versatility while responding to biohazard risk analysis as well as to requirements for biosafety compliance, validation, safe operation and ergonomic use. A comprehensive approach is required to include all these requirements.
Biosafety containment requires the combined use of adequate biosafety equipment (primary containment), secondary biocontainment, personnel protective equipment and suitable working practices (including education and training). All these requirements must (i) respond to a biohazard risk analysis (combining the infectious agent(s) and the expected uses) and its related mitigation plan, and (ii) be strictly, consistently and profes-

sionally implemented, with suitable training, monitoring and records management. The design of classical biosafety cabinets (BSC type I, II and III) and their constraints of use do not meet all specific requirements of rodent-based research, both for housing and for care & use procedures. This situation led to the development of innovative IVC-based biocontainment solutions, responding to the specific and integrated needs of scientists and animal technicians, as well as to the regulations and best practices expressed by OHS / Biosafety Officers and Quality Management. A new generation of IVC cages / racks and cabinets, specifically designed to meet these needs, allows responding in a comprehensive way to risk analysis and validation processes, to overall safe operation combining the use of IVC biocontainment units and purpose-designed biosafety cabinets

OC1S1 The 3Rs and animal models involved in immunology research

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UCB Pharma

Immunology research is a broad area covering chronic inflammation, autoimmune diseases and transplantation. From a research perspective, robust and reproducible models are required enabling the study of immune mechanisms relevant to human disease.
A wide range of models exist to fulfil this goal from short-term mechanistic models looking at inflammatory cell recruitment through to chronic disease models, in which there is a complex interplay between the different arms (innate and adaptive) of the immune response and with disease pathology in a relevant tissue compartment. However, the disease aetiology is different and animal models do not recapitulate all features of the human disease with only a subset of disease signs being displayed and multiple models may be needed to replicate all components of the human disease. Nevertheless, animal models in immunology research are amenable to the 3Rs particularly reduction and replacement The use of in vivo screening cascades, appropriate group sizes, Bayesian statistics can lead to a reduction in the number of animals used. Refinements can be made to models, which reduce the burden on the animal whilst ensuring high quality experimental data is generated. Examples will be discussed in this presentation.

OC1S2 3Rs in vaccines manufactures: a strategic focus, more than a moral and legal obligation

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Vaccination is one of the most effective medical intervention ever introduced allowing saving billions of lives in the word. To be able to bring this human health benefit, the vaccines industry is still using large numbers of animals. In 2011, De Mattia et al estimate it to be about 15% of total animal use in biomedical research and testing.
The principle of the 3Rs — Replacement, Reduction and Refinement – is a widely accepted framework that can be used for ethical and scientific review in conducting scientific experiments using animals humanely. This principle is embedded in most legislation worldwide and particularly in the European Directive 2010/63/EU. More than a legal and ethical obligation, 3Rs is for the vaccines industry a strategy priority. The presentation will focus on how the human vaccine manufacturing industries have integrated since decades the principal of the 3Rs in all the dimensions of the vaccines process and have turned this strong commitment on 3Rs into a strategic priority. GSK as a global company is committed for more than 20 years to adherence to the principles of the 3Rs. In Vaccines and in the other GSK divisions, animal work remains a small but vital component of our Discovery and Development process and is mandated by different laws. GSK is committed to aligning its strategy for animal work with societal expectations of meeting regulations, performing well-designed studies, practicing a culture of care and concern for animals and vigorously pursuing advances in sciences that allow us to maximize the value from the models we use and replace animal models where possible. The Vaccines division has used laboratory animals for Research & Development to understand disease patterns, assess preclinical immunogenicity, efficacy and safety of candidate vaccines. In some rare occasions, Vaccines division also uses laboratory animal cells for production of the vaccines. But one of the highest uses is related to the batch release activity as per legal obligation. The presentation will briefly review the use of laboratory animals in vaccines, describe how the 3Rs principles as well as their constraints and advantages can be considered as a strategic opportunity for GSK Vaccines. The presentation also highlights the importance of senior leadership engagement and support, communication and training, Project management. Additionally, collaboration internally and externally with other industries and public institution are key elements to turn the strategy into tangible benefit for human health, society expectation, patient benefit, sciences, quality as well as better lean manufacturing process to support global heath.

OC1S3 Comparative cytokine and Toll-like receptor (TLRs) gene expression profile in mucosal-like mast cells (MLMC) derived from B6.129P2-Il10tm1Cgn/J (IL-10 KO) and C57BL/6J (WT) mice

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Interleukin-10 KO mice (IL-10 KO), is a common model to study inflammatory bowel disease (IBD) because they present spontaneous colitis. Mast cells have also been associated with this disease and IL-10 is a cytokine implicated in the differentiation of mucosal mast cells. In this context, IL-10 KO model was used to determine the gene expression of cytokines and TLRs in mast cells and to compare with wilde-type mice before and later development of the disease and under stimulation with LPS
Material and methods: To do this, bone marrow was obtained from femur and tibia of B6.129P2-Il10tm1Cgn/J (IL-10 KO) and C57BL/6J (WT) mice (The Jackson Laboratory, Maine, USA) of 6 and 20 weeks old and then proceeds to its culture and differentiation to mucosal-like mast cell phenotype in DMEM/FCS (Life Technologies Ltd, Paisley, UK) supplemented with recombinant mouse IL-3 (rmIL-3), rmIL-9 (R&D Systems, Abingdon, UK), rmSCF (Peprotech, London, UK) and rhTGF-β1 (Sigma, St. Louis, MO), this combination of cytokines is referred to as TI3S, as described by De Jonge et al., 2004. After 9 days of culture, 5x105 cells/mL of MLMC, were harvested (time 0, unstimulated cells) or stimulated with different concentration of lipopolysaccharide (LPS) from Escherichia coli 055:B5 (1,10, 100 and 1000 µg/mL) and then harvested at 6 or 24 hours. To analyse the gene expression profile of cytokines (TNF-α, IFN-γ, IL-1β and IL-6) and Toll-like receptors (TLRs) (TLRs-2, 4, 6, 7 and 8), first we extracted the total RNA from harvested cells using the RNeasy Mini Kit (Qiagen), then proceed to the reverse transcription using iScriptTM cDNA Synthesis kit (BIO-RAD) according to the manufacturer's specifications. Finally, the PCR reaction was carried out using pre-designed Taq-Man probes for each gene over a C1000 Touch™ Thermal Cycler platform (BIO-RAD). Finally the data analysis of relative gene expression data was done using the GraphPad Software applying 2-ΔΔCT Method. Results: Through analysis by RT-qPCR, we found that at 6 weeks and time zero (unstimulated) there are no differences in the expression of TNF-α, IL-6, IFN-γ, IL-1β, TLR-2 , 4, 6, 7 and 8 between WT and KO animals; however, at 20 weeks (time zero, unstimulated) significant differences were detected in the expression of IFN-γ, TLR-4, 7 and 8. Moreover, most of the analysed genes, except TLR-4, show significant differences between WT and KO by stimulating with LPS at different times. Conclusions: Results show that either prior to the development of the disease or later, there are differences in the gene expression profile of mast cells between KO and WT mice under stimulation, which is interesting to investigate about the differentiation and response of the mast cell in disease condition. These differences may allow understanding the mechanisms for the development of IBD and its treatment. Results also contribute to the better knowledge of this experimental model.

OC2S1 Development of a new method to examine the therapeutic potential of non-dopaminergic drugs in the unilateral 6-OHDA-lesioned rat model of Parkinson's disease

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The motor symptoms of Parkinson's disease (PD), i.e. bradykinesia, resting tremor, rigidity and postural instability, mainly result from the progressive loss of dopaminergic neurons in the substantia nigra, leading to dopamine (DA) deficiency. While DA replacement therapy is the gold standard for treating PD, the use of L-Dopa or DA agonists is also associated with motor complications, i.e. dyskinesia, wearing-off. Preclinical rodent models reproduce different aspects of the pathology.
The gene encoding α-synuclein (SNCA) has been identified as causing autosomal-dominant forms of PD. The deposit of aggregated α-syn in degenerating neurons is the pathological cellular hallmark of both genetic and idiopathic PD. The disease-gene based models, especially α-syn overexpressing models, are currently suited for the development of disease-modifying therapies and for understanding the causes of the illness. These models are progressive and reproduce numerous molecular and cellular aspects of α-syn pathology. However most of them lack sufficient DA neurodegeneration to induce motor deficit. The toxin-based models are not progressive but exhibit the advantage of reproducing the typical DA degeneration and the motor phenotype observed at the time of PD diagnosis. These characteristics make them unique for the evaluation of compounds targeting the restoration of motor activity and/or the prevention of motor complication. The unilateral 6-OHDA-lesioned rat model has been a reliable workhorse for more than four decades. It consists of the unilateral brain administration of a toxin, 6-hydroxydopamine, which induces a strong degeneration of the dopaminergic system in one brain hemisphere (i.e. reduction of striatal dopamine and nigral tyrosine hydroxylase). A behavioural phenotype is noted in the body side contralateral to the lesion. The most widely used locomotor assessment for hemilesioned rats is the circling response. The lesioned rat spontaneously orientates its behavior towards the damaged side (ipsilateral exploration). Drugs that stimulate dopamine receptors cause the rat to turn in a direction opposite to the lesioned side (contralateral rotations).The objective of this work was to refine the unilateral 6-OHDA-lesioned rat model to optimize its use for phenotypic screening and testing new drugs. Procedures were standardized to obtain reliable and statistically significant data with a minimal number of animals. Several methods were implemented to improve the variability (surgery, injection, timelines, selected randomization, behavioural analysis, handling, animal inclusion criteria). A new behavioural analysis based on videorecording and automated scoring of behaviour was implemented. The aim was to capitalize on the existing data and further develop the use and understanding of the hemilesioned rat model. Thanks to this setup, a new method specific for the screening of potential non-dopaminergic drugs was implemented and standardized.

OC2S2 Non-Human Primate research to support development for novel pharmacological treatment of Parkinson's Disease

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Of the clinical candidates that enter phase 1, approximately one out of ten is approved by FDA. This low success rate is very concerning for drug developers, regulators and patients. We present an overview of Non-Human Primate (NHP) models that help selecting clinical candidates, their dose and expected efficacy for Parkinson's Disease (PD).
In order to determine the absolute concentration of drug candidates, we developed a microdialysis technique (MetaQuant) that allows determination of absolute free drug concentrations in the brain. This method is shown to provide more reliable data, rapid turnover and results in a significant reduction of animals needed compared to classical testing. Furthermore, this method is being applied in many different species including rodents, guinea pigs and non-human primates (NHPs). With microdialysis, neurotransmitters and larger molecules (e.g. α-synuclein) can be measured in order to confirm the mechanism of action. However, using conventional bioanalytical techniques, multiple neurotransmitters cannot be quantified in the same sample, hence, large animal numbers are needed. Therefore, we established a method that allows analysis of up to 16 different neurotransmitters in a single sample. With this method the number of animals can be further reduced by 33% and still making measurement of the full neurotransmitter panel possible. Finally, we apply the same approach to analyse blood, brain and cerebral spinal fluid (CSF) samples, to get a full overview of pharmacokinetic / pharmacodynamic effects from the same awake animals on PD relevant markers in these three compartments.
In addition to pharmacokinetics and pharmacodynamics in naïve animals, the use of PD models is important to evaluate the efficacy of drug candidates in models with construct and face validity. Toxin models such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are frequently applied in NHPs. As an example, the 6-OHDA marmoset model is suitable for studying neuroregeneration and tolerability of neurotrophic factors such as cerebral dopamine neurotrophic factor (CDNF) by using magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) and immunohistochemistry. In addition, new models that focus on the protein α-synuclein, such as viral vector-mediated overexpression of human α-synuclein or intracerebral injections of oligomeric and fibrillary forms of α-synuclein, allow a more etiological insight into the disease process. However, due to the slow disease progression this approach takes very long compared to classic models. Here we discuss advantages and disadvantages of available and newly developed models of PD in NHPs.

OC2S3 Behaviour and neurophysiology in the validated MPTP marmoset model for idiopathic Parkinson's disease

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Aging societies face an increasing prevalence of neurodegenerative disorders like Parkinson's disease, for which no cure exists. The paucity of relevant animal models that faithfully reproduce clinical and pathogenic features of neurodegenerative diseases is a major cause for the lack of effective therapies.
Our closest relatives, non-human primates, and in particular the marmoset monkey, provide an appropriate animal model for construct, face and predictive validity owing to the close anatomical and physiological proximity to human. The well-established 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of idiopathic Parkinson's disease in marmoset monkeys (Callithrix jacchus), recapitulates most of the core pathogenic mechanisms of the human condition. Repeated injections of the neurotoxin MPTP create a valid preclinical Parkinson's disease model caused

by progressive apoptotic cell loss of dopaminergic neurons in the substantia nigra, resulting in the characteristic symptomology of Parkinson's disease, i.e. bradykinesia, tremors and rigidity and the typical motor dysfunction and non-motor signs, such as sleep problems. Since sleep disturbances generally become apparent in Parkinson's disease before motor symptoms emerge, they may represent early diagnostic tools and a research tool to investigate early pathology and disease modifying strategies. In my presentation, I will give an overview of different non-invasive behavioural and neurophysiological applications in the marmoset model for Parkinson's disease, including sleep electroencephalogram (EEG) using telemetric techniques for remote monitoring of brain activity in unrestrained monkeys. Home cage testing facilitates long term monitoring of performance alongside collection of neurophysiological data by telemetric means with minimal disturbance to the free moving animal, which fits within the three R-policy at the Biomedical Primate Research Centre BPRC.

OC3M1 Monitoring rodent behaviour in a home cage environment: automation enhances research quality, efficiency and animal welfare

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Efficient and reliable assessment of rodent behaviour is key to successful basic research and preclinical drug discovery and development. However, traditional behavioural tests, carried out in a wide variety of dedicated apparatus, are time-consuming and their reproducibility is confounded by factors such as human-animal interaction and the lack of standardization in test apparatus and protocols.
Recent advances in computer vision and sensor technology, and our increasing ability to process large data sets, are bringing about unprecedented possibilities to analyse animal behaviour. These developments also greatly contribute to better animal welfare, both directly and indirectly. Systems have been developed for automated monitoring and analysis of rodent behaviour in a home cage environment, i.e. a cage suitable for prolonged housing of a laboratory animal and equipped with sensors and stimulus devices allowing automated measurement of spontaneous behaviour and cognitive performance. These systems facilitate measuring behaviour in a standardized setting, without human intervention, for prolonged periods of time, from hours up to multiple days or weeks. This not only increases efficiency, it also limits stress of handling. Because tests are performed in 'the comfort of a home cage', the negative effect of transportation and a novel environment on both the wellbeing and the performance of the animal in the test is eliminated. Some standard tests such as novel object recognition can easily be adjusted to a home cage environment. Other tests such as the Morris water maze paradigm to assess learning and memory can be replaced by novel paradigms that address the same brain function but are compatible with a home cage environment, while being less stressful for the animal. An example is the CognitionWall™, a device and corresponding test protocol designed for the PhenoTyper® cage, which has proven to yield data at least as valuable as the water maze. The combination of automation, objectivity and large datasets greatly enhances the efficiency, sensitivity and reproducibility of behavioural experiments, including general behavioural phenotyping of mouse and rat mutants and in vivo efficacy testing of drugs for psychiatric and neurodegenerative diseases. Furthermore, the technique can also be used for continuous monitoring of animal wellbeing and the early detection of disease. Summing up, automated behavioural assessment in a home cage environment refines tests, reduces the numbers of animals and tests needed to achieve the same statistical power, and replaces tests that would normally be carried out in novel and possibly unpleasant environments for the animal.

OC3M2 Experiences from using sensor technology in measuring behaviour for production animals at farm level and in the production chain

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Management of production animals like cows, pigs and poultry nowadays is supported by 'real time' data from the production. Measuring behaviour of individual animals that are part of a group is used for optimizing production and early warning of health problems. These behavioural data can be generated and used in different parts of the production chain.
In this presentation we will give insight in the use of sensor technology in the dairy and poultry sector and give some examples. We will address specific drivers for development and innovations in sensor technology. One of these drivers is animal welfare, but there are more drivers. We will also address some of the success and failure factors when this has to be implemented in real farming situations. Success in quantifying welfare, production and behaviour and contributions to transparency will be discussed. Still there is a lack of use in using this behavioural data for practical work instructions. For that you need to be able to look at multivariant issues and should have knowledge of the context. Main focus will be on farm level, but data are also gathered and used in the processing plants e.g. foot pad lesions are measured at the slaughter line instead of in the broiler house.

OC3M3 Physical basics of the Electromagnetic Fields (EMF) that surround us; what are they, how do they occur and where are they? Can this phenomena be used to help with our culture of care?

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This presentation is for technicians, managers and facility veterinarians to help them understand about the basics of Electromagnetic Fields (EMF) and what the sources are. New technologies in animal environments are continually increasing and evolving which introduces new sources of EMF. These technologies allow improved communications, staff efficiencies in animal care and the quality of animal care, and ultimately improve animal welfare. EMF effects on humans and animals will be discussed.
Electromagnetic Fields (EMFs) are part of life and we are immersed in them. This presentation will cover what EMFs are and where they are, as well as how they are different from Radiofrequency fields (both which are commonly present in the animal holding rooms). Low Frequency Electromagnetic Fields (LEMFs) will be differentiated from other EMFs types. Different frequencies (HZ), Electric fields (V/m) and Magnetic flux densities (uT) will be reviewed. A typical animal holding room with standard equipment will be characterized as to the types of EMFs emitted and how they are characterized. As vivarium's increase in sophistication, the use of modern technologies (cell phones, lap tops, computers, RFID systems, behavioural analysis equipment (video cameras, pressure pads, IR beams, and EMFs)) in the animal environment is more pervasive. These technologies improve data capture, communications and staff efficiencies thereby increasing the quality and quantity of the science, animal care

and ultimately modify outcomes of animal welfare. EMFs are continually around us, and, as the use of technology increases so does the EMFs in the animal areas. Since we are using more technical equipment in closer proximity to the animal's home cages potential animal exposure levels will be discussed. A brief review of the known hazards associated with the different characterizations of the EMFs will be presented. Since the EMFs are continually present, one should wonder if this physical component could be used to help us monitor the animal environment as a non-invasive home cage technology to improve scientific data and animal welfare (our inherent culture of care)? This question will be reviewed at a high level, and current practices of evaluating the science will be discussed.

OC3M4 Exposure of mice to low intensity EMF

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The recent development of rodent cages equipped with sophisticated technologies using low intensity Electro Magnetic Fields (EMF), in the range of 5 Hz to 100 Hz (for the purposes of continuous monitoring of both the behaviour/welfare of the animals and a number of cage environmental parameters) raises questions on the potential effects of EMF on the animals, even though EMF are generally considered to have low biological activity.
Materials and methods. The aim of this study was to perform a long term (up to one year) clinical-pathological study of mice exposed to continuous EMF at very low intensities. Three-hundred twenty male and female C57Bl/6N mice were randomly divided into control and exposed groups on a single IVC rack. Throughout the experiment, body weight, water and diet consumption were recorded at 14 day intervals. At sacrifice (programmed after 60, 120, 180, and 365 days of exposure) haematology, bone marrow analysis, and histology on major organs was performed. Results. At all examined time-points no significant alterations of body weight, water and diet consumption between exposed mice and the controls were detected. Haematology, bone marrow and histology were within normal limits. Discussion and Conclusions. Long term, continuous exposure to low level EMF showed no relevant clinical-pathological effects on mice.

OC4S1 AWAG: an easy to use software system for assessing the lifetime experience of animals

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Public Health England

Public Health England and University of Surrey have developed an easy to use software application known as the Animal Welfare Assessment Grid which tracks the life-course of animals used in facilities and produces a graphical representation of welfare data. The system has been designed to record the impact of numerous parameters such as housing, social grouping, transportation and experimental procedures, such that the lifetime experience of different species can be quantified.
A software package (AWAG) has been developed by Public Health England to provide an easy to use assessment system that can record and depict the combined effects of environment, physical and psychological well-being and contingent events on welfare. The system has been validated for use in experimental macaques (Wolfensohn et al, 2015) and is now being piloted in other primates at Marwell Zoo, UK. The grid examines the welfare of animals at key points throughout their life, taking into account the duration as well as the intensity of suffering and produces both numeric and visual presentation of the animals' welfare at each point in time. By scoring four parameters (Physical, Psychological, Environmental and Procedural) which encompasses the five freedoms, this system develops a matrix which uses data collected as an intrinsic part of experimental and husbandry records. The AWAG 's temporal approach will allow those caring for animals to plan or intervene with targeted and timely refinements that can improve or prevent deterioration of an animal's quality of life. The AWAG enables retrospective review by welfare bodies by visualising an overview of welfare during the conduct of a project but also allows any reviewer to drill down to identify specific items that have had a negative effect on welfare. Data captured by the AWAG can enhance the culture of care at an establishment by empowering persons responsible for welfare through the provision of evidence that is easily understood by all parties involved in project planning or funding. The software system is now available for free download via an open source website and is a web-based application designed to be installed on an organisation's IT infrastructure. A number of examples of how welfare of animals housed under high level biocontainment has been assessed and improved will be given.

OC4S2 Validation of a novel biomarker of cumulative experience of non-human primates involved in biomedical research

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Researchers have ethical and legal obligations to optimise the physical and emotional wellbeing of their animals. Furthermore, current European legislation places an emphasis on the animal's lifetime experience (1). However, current methods for assessing the cumulative experience of animals are poorly validated and suffer from a lack of sensitivity and/or specificity. The aim of this work was to develop and validate a new method to assess cumulative experience in non-human primates (NHP).
Materials and methods: Artificial weaning is a well-established early-life stressor in NHP. It is also known to have long-lasting detrimental effects on emotionality, social, sexual and maternal behaviours, growth, immune responses and in some cases survival (2). Individuals weaned early are thus likely to have a poorer life time experience compared to individuals weaned at an older age. As a consequence, one would expect any sensitive bio-marker of cumulative experience to be influenced by weaning age. Experimental evidence recently accumulated in humans, NHP and rodents suggests that the amount of grey matter in the anterior hippocampus might be a marker of cumulative experience (3). Using neuroimaging techniques (MRI), we quantified non-invasively the amount of grey matter in the anterior hippocampus of adult male macaques (N = 12) and tested the hypothesis that an early weaning age leads to less grey matter in the anterior hippocampus. Results: After controlling for covariates including age and brain size, a multiple regression analysis revealed a positive correlation between amount of grey matter in the right anterior hippocampus and weaning age (range: 6 to 33 months).Discussion and Conclusion: This result supports the idea that the amount of grey matter in the anterior hippocampus is a sensitive biomarker of cumulative experience in NHP. This novel biomarker can now be used to identify and refine the experimental and/or husbandry procedures having a detrimental effect on the emotional well-being of non-human primates. The weaning

age currently recommended for macaques is 10-12 months2. Our data suggest that a later weaning age might be beneficial for the monkeys. We will formally test this specific hypothesis in the near future. Because the hippocampus is a brain structure extremely well conserved across taxa, a similar approach could be used to measure cumulative experience in many species used in bio-medical research, including rodents.

OC4S3 Refinement in NHP models: An innovative automated tool to assess cognitive abilities in group-housed macaques

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In neurosciences, behavioural studies are crucial in that they can measure the visible part of cognitive processes. Studying the evolution of subjects' behavioural phenotypes through specific neuropsychological tasks has already demonstrated its efficiency (Weed et al. 1999). In our study, we combine this approach with a new automated apparatus allowing subjects to be tested in a non-invasive way when they want while staying in their social group.
NHPs are able to practice different paradigms used in humans and to learn several tasks which make them a key model for studying normal or altered cognitive functions. Our experimental device is designed to accurately measure cognitive performances in monkeys living in social groups. It involves operant conditioning only, without deprivation or physical constraint. Animals have a free access to the device thanks to an automated identification system (Fagot et al 2009). Tests are provided via a touch-screen interface, as in humans. The original features of our approach lie in the implementation of a new learning process of different cognitive tasks. The difficulty of each task changes in real time according to individual performances so that the full experimental device operates autonomously. We are currently studying cognitive performances in 3 species of macaques (M. mulatta, M. fascicularis and M. tonkeana) in several tasks targeting working memory, visual short-term memory, conditional memory, and attention. When reaching stable performances, some subjects receive a dose of scopolamine, a reference compound impacting memory and attention (Taffe et al. 1999). This is to evaluate the drug effect in tasks performances and validate the relevance of the system. The experimental device appears very attractive for the macaques who use to perform several hundred trials per day. Through this new approach totally automated and autonomous, subjects can learn and perform different cognitive tasks in parallel. Learning processes have been optimized, so that subjects are able to obtain high performances in just a few days. Then, performances stayed perfectly stable over time. The attentional and the inhibitory control performances seems particularly high through this non-invasive approach compared to the results obtained through classical approaches on isolated monkeys. The patterns of results obtained in subjects that differ in age show obvious differences regarding to learning capacities and maximal performances on visual, working and conditional memories. The system appears very powerful to reveal specific differences in animal cognitive abilities and relevant to conduct comparative studies. Results will be discussed in the light of the interest of such a tool for studying underlying processes of cognitive functions as well as the application of this standardized method to evaluate drug effects on learning process, memory and attention.

OC4S4 The institution implemented a guidance document for the use of scientists, technicians and animal care personnel in order to implement a harmonized approach to report the actual severity assessment of the animals

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We worked on a guidance document to harmonise the way that actual severity is recorded within GSK sites that are under the European Directive 2010/63/UE. The aim of the guidance is to help individuals that are asked to assess the actual severity of animals under study and to be aware of how they should classify this.
The prospective classification of an experimental procedure predicts the worst severity that could be expected during the procedure. An actual assessment needs to take into account what really happened and it's often the case that the actual severity is lower than what was predicted. When the Directive (1) describes the severity level of a specific model or procedure this severity level will be applied to such protocols. We also aligned the document to the recommendations of the commission to harmonise the European statistics as they are pertinent to those involved in the assessments. Mistakes in classification can occur e.g. a health problem has a different classification if it is due to the protocol rather than an external factor. This work aims to give more precise guidance in areas where the Directive is not prescriptive. Use of this guidance will make the animal usage data collected for GSK internal reports more comparable and will enhance the accuracy of the European statistic reports. Many examples of models or procedures currently in use have been discussed and included in this guidance. Some inter-country differences on how the Directive is interpreted have been identified and are highlighted. The examples presented here aim to support the process of severity classification, however, this guidance does not intend to replace professional judgement of those responsible for assessing severity.

OC5W1 Classification and Reporting of Severity - an interactive workshop to examine the process and challenges of achieving consensus

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Directive 2010/63/EU introduced the requirement for the classification of procedures (Article 15) during the application for project authorisation to use animals in scientific procedures. It also introduced the requirement to report the actual severity experienced by each animal used in a procedure. Both these processes provide opportunity to refine the adverse effects of procedures.
Consistency of assignment of severity categories across Member States is a key requirement. The examples given in Annex IX are limited in number and have little descriptive power to aid assignment. Additionally, the examples given relate to the procedure and do not attempt to assess the outcome, such as adverse effects that may occur. The aims of this interactive session are to discuss the challenges faced by the FELASA/ECLAM/ESLAV Working Group that has taken a number of current animal models to illustrate the severity process from inception of the project, through

monitoring during the course of the procedure, to the final assessment of actual severity at the end of the procedure. The impact of refinement on the potential adverse effects and consequence to the assigned prospective severity will be highlighted. The session will commence with an introduction to the severity framework .Using a model from the FELASA/ECLAM/ESLAV Working Group, each group of participants will identify procedures involved, define adverse effects, identify actions to mitigate the adverse effects, identify appropriate end points and finally assign a prospective severity classification. Each group will define what clinical welfare assessment criteria should be used and how to classify the actual severity experienced. Each group will present their findings and individual turning point responses will be used to gather views/promote discussion.

OC6S1 The perennial debate on applying animal welfare standards to fish and the implications for fish husbandry and procedures

Schroeder, Paul, Presenting author
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Discussions on fish sentience were first sparked by Verheijen's seminal work on post hooking behaviour in carp in 1983. With the work by Lynne Sneddon and those who followed suite, on nociceptors and on post-nociceptive behaviour in a range of teleosts, there is increasingly robust evidence base supporting the concept of a state akin to what we might regard as pain and fear in fish.
EU and UK statutory controls of animals used in scientific procedures have endorsed this evidence. At the same time, a number of researchers across the globe have challenged the findings, proposing that the concept of a higher conscience in fish is rooted in the anthropomorphic extension of the human understanding of pain and fear. With few exceptions the side opposing the idea of the existence of pain in fish has either been funded by or closely linked to the recreational fishing lobby. They also have not produced a single piece of original evidence and have been confined by the simplistic dogma that structure defines function, in that their lack of a neocortex makes fish incapable of feeling pain. At least in the UK, this debate has stalled the progression of welfare improvements for fish in research facilities. Fear of compromising research results and the absence of published practical analgesic protocols suitable for small fish, for example by immersion, can push the researchers into the embracing entrenched view that fish do not have the anatomical prerequisite to experience pain and that all attempts at giving fish analgesia are a waste of time.

OC6S2 Exploring suitable analgesics in zebrafish - a combined approach

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Home Office guidelines recommend the use of analgesics in all scientific procedures likely to result in pain, suffering and lasting harm for all protected species, including fish. Perioperative analgesia administered intramuscularly or at the site of tissue damage has shown efficacy in larger fish species for some drugs. However, this is impractical in very small species (<1g) and when large numbers need to be treated.
Immersion is a commonly used route of administering anaesthesia in fish and may be a less invasive alternative to administering analgesia. Therefore, this study examined the efficacy of three water soluble analgesic drugs (buffered acetylsalicylic acid, butorphanol tartrate and lidocaine) provided pre- and post-surgery (tail fin clip) through addition to the tank water. The effectiveness of these drugs was measured as the reduction of changes in pain-related post-surgical behaviour and since pain is inherently stressful, HPI axis activity, quantified as whole body cortisol levels (determined through radioimmunoassay). To determine uptake, whole body drug residuals was determined through HPLC-mass spectroscopy for each drug. Both lidocaine and acetylsalicylic acid resulted in a significant reduction of pain-related behavioural patterns, compared with animals just given a placebo (saline). There was no effect of treatment on whole body cortisol. Finally, uptake was verified for lidocaine showing clear dose dependency, while the other two compounds were under the detection threshold of the mass spectrometer. As the only drug with both a behavioural sparing effect and pharmacokinetic validation in this study, low-dose lidocaine immersions can be recommended for the first time to aid perioperative analgesia and provide a valuable refinement for reducing the impact of fin clipping in zebrafish thereby improving welfare.

OC6S3 Bringing the 3Rs to the zebrafish

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In the last few years, some 3R applications were developed to improve our care and procedural use of zebrafish. Though there is a gap between a good idea and an actual refinement in practice - moreover when the understanding of the species is poor.
We will explain here the pitfalls and the positive outcomes from our exploration of how to implement the 3Rs in the laboratory zebrafish. This will start with the efficacy of mucus swabbing as an alternative to fin clipping. Then we will consider the averseness, potency and toxicity of various anaesthetics and we will explain the rationale for good practice in anaesthesia and euthanasia. Adding devices and decorative items to the tank and its surrounding is also suggested to improve the environmental enrichment of zebrafish and we will evoke what we learnt from exploring this route in order to improve our breeding set-up for example. Another major topic for discussion is feeding the zebrafish better but with less human interventions. We will detail the parameters we learnt to monitor when studying various diets and their performance regarding growth, fertility, husbandry as well as practicalities and biosecurity. Finally, the fish told us what they preferred and it is by observing them again and again that we started monitoring morbidity and mortality. This should be routine practice to monitor system failures or transgenesis adverse effects.

OC6S4 Relevant environmental enrichment for laboratory fish - a review

Schroeder, Paul, Presenting author
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Over the last five years there has been a steady flow of publications addressing function and utility of environmental enrichment for fish. Despite this, in the joint FELASA/COST working group's deliberations on zebrafish housing, husbandry and health monitoring, no recommendations were made with regard to this important subject matter.
Environmental enrichment comprises a structural, social and feeding element (such as live feeds). Studies have mainly focused on structural enrichment as social enrichment was only included as control cue for structural enrichment. Feeding enrichment has not been investigated at all except as an additional treatment group in structural studies. To date, publications have either focused on the absence/presence of preference

for structurally enriched environments or on the effect of enrichment on behavioural and physiological outputs. A number of publications also explored how preference is modulated by the animals' social context (social, pair or single housing). A number of principal trends have been substantiated: Naturalistic tank additions are significantly preferred by a range of species when pitched against barren tank compartments. The long term effect of subjecting captive fish to these enrichments, in terms of impact on stress physiology and behaviour, is variable and may also depend on the age at which these species are first exposed. On the whole, these results may be considered not conclusive enough to facilitate substantial buy-in into the concept of providing environmental enrichment. This study therefore identifies a number of commonly applied structural enrichment building blocks (substrate, artificial plants, shelter, enrichment pictures), evaluating their relevance from the outcome of preference testing, behavioural and physiological investigations as well as biosecurity considerations.

OC7M1 Humane end points: help or hindrance

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Humane intervention applied to in vivo medical experiments can take many forms.
Firstly, culling maybe independent of the experiment for example when animals have been fighting. Secondly, culling may be due to technical failures, and whilst every effort should be taken to minimise loss of animals in this way, losses can be allowed for in selection of group sizes so that statistical analysis is not compromised. In both examples animals lost are not good experimental subjects and science is improved as a result of their removal. In a third instance it may be necessary to intervene if severity of an experiment crosses pre-agreed welfare boundaries. What to do with the data from these animals is an issue scientifically, as removal of the data skews the results, and carrying data over is a compromise that is increasingly unsatisfactory the longer the study. But it is not the humane end points themselves that are the problem. If there is an absolute requirement for severe disease then humane end points should be applied that are consistent with the experimental aims. But usually severe disease is not an experimental requirement and work can be conducted to change protocols to lessen the disease burden. Scientists need to justify the level of disease severity at ethical review and humane end points set that are consistent with the experimental aims without allowing suffering beyond what is necessary. Set appropriately, against a background of refined experimental techniques, humane intervention should be unusual and therefore not a hindrance to good science.

OC7M2 Assessing the potential for pain in invertebrates

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All animals face hazards that cause tissue damage and most have nociceptive reflex responses that protect them from such damage. However, some taxa have also evolved the capacity for pain experience, presumably to enhance long-term protection through behaviour modification based on memory of the unpleasant nature of pain. In this talk I review various criteria that might distinguish nociception from pain.
Because nociceptors are so taxonomically widespread, simply demonstrating their presence is not sufficient. Furthermore, investigation of the central nervous system provides limited clues about the potential to experience pain. Opioids and other analgesics might indicate a central modulation of responses but often peripheral effects could explain the analgesia; thus reduction of responses by analgesics and opioids does not allow clear discrimination between nociception and pain. Physiological changes in response to noxious stimuli or the threat of a noxious stimulus might prove useful but, to date, application to invertebrates is limited. Behaviour of the organism provides the greatest insights. Rapid avoidance learning and prolonged memory indicate central processing rather than simple reflex and are consistent with the experience of pain. Complex, prolonged grooming or rubbing may demonstrate an awareness of the specific site of stimulus application. Trade-offs with other motivational systems indicate central processing, and an ability to use complex information suggests sufficient cognitive ability for the animal to have a fitness benefit from a pain experience. Available data are consistent with the idea of pain in some invertebrates and go beyond the idea of just nociception but are not definitive. In the absence of conclusive data, more humane care for invertebrates is suggested.

OD1S1 Cephalopods as Laboratory Animals: from classical 'use', to Guidelines and towards mandated minima

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The inclusion of cephalopods in Directive 2010/63 represents a landmark. It is the first time that an entire class of invertebrates (700 species) is included in laboratory animal legislation in EU. Their inclusion is based on precautionary principle for their capacity to experience pain, suffering, distress and lasting harm. Directive provides general guidance for species covered, but information for cephalopods is missing.
We coordinated a community effort to foster the 'transit' from un-regulated to regulated framework at the EU level. We first developed "Guidelines for the Care and Welfare of Cephalopods in Research, a consensus based on an initiative by CephRes, FELASA and the Boyd Group". Guidelines are the result of an international initiative aimed to collate information for the care and welfare of cephalopods (e.g. nautilus, cuttlefish, squid and octopus) in compliance with the Directive. These provide information for investigators, animal care committees, facility managers and animal care staff improving both on care and procedures applied to these animals. Topics included: implications of the Directive for cephalopod research; project application requirements and authorization process; the application of the 3Rs principles; the need for harm-benefit assessment and severity classification. General species-specific requirements are discussed on: i. supply, capture and transport; ii. environmental characteristics and design of facilities; iii. accommodation and care, animal handling, feeding and environmental enrichment; iv. assessment of health and welfare; v. approaches to severity assessment; vi. disease; vii. scientific procedures, and general anaesthesia and analgesia, methods of humane killing, confirmation of death. In addition, we will discuss how the COST Action FA1301 deal with these topics and is promoting the definition of species-specific mandated minima for care and welfare of cephalopod species in respect of the principles stated in the Directive 2010/63/EU. We will provide suggestions and facilitate discussion about research priorities including the evaluation of cephalopods well-being and health status, thus to facilitate researchers, veterinarians and regulators to deal with the novelty of cephalopods as 'marine guinea-pigs'. Our review is based on the outcomes of the last five years of networking activities including those carried out under the aegis of the COST Action FA1301 – CephsInAction "A network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries". This COST Action operates under H2020 principles of growing ideas through networks and COST Policies and is aimed to facilitate and challenges that the scientific community working with the first regulated invertebrates are currently dealing with.

OD1S2 Training and certification for the collection of wild cephalopods

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Cephalopod supply as model animals for laboratory research is currently reduced. For a number of years a now discontinued facility provided purpose bred animals for researchers, particularly in the medical field. In replacement only a very few species, notably in the family Sepiidae, are currently available, while demand is unabated. The need for training and certification to collect animals from the field within the remit of the regulation on care and welfare of these taxa is therefore evident.
METHODOLOGY Specific training schools on care and welfare of cephalopods have been launched, where early career researchers, veterinarians and laboratory technicians were provided with theory and practical training on regulation and best practice for cephalopod care and welfare. Fishers in Portugal and Italy were simultaneously informally contacted on possible interest on training directed at the collection, maintenance, transport and basic caring for a number of species commonly exploited in fisheries, with a view to replace some fisheries kills with animal supplies for laboratory research. RESULTS Interest from small-scale fisheries stakeholders is preliminary guaranteed, as are the requirements of the training to be delivered and the body of staff that will be required. Trial sessions demonstrated the feasibility and determined the conditions that will be necessary for training to be taken to different people of specific language requirements and skill levels. The training and certification mechanisms that are necessary have been established. DISCUSSION AND CONCLUSIONS Knowing the importance of cephalopods as research 'models' and the implications that their availability may have for the advancement of knowledge, makes a strong case for the development of conditions that may make the animals predictably available. On a par is the importance of the welfare of the animals that will be used in research. Therefore, a prime concern must be the development of a process through which quality training can be continuously provided, and a system of certification created, that will ensure that those people directly involved in the putative collection of the required specimens, can guarantee their basic care and welfare, from the wild to the supply intermediary or the destination laboratory. This must also include knowledge on the ecological status of the populations and therefore requires that the species that are made available are the same that are studied for their fisheries value. A corollary is that the objectives of this training cannot be to encompass any species, but simply those with a well-known conservation status, and therefore that a priori this list will not be closed and can change both with the natural conservation status of the donor populations, and with the level of the existing expert knowledge. The creation of such a system is currently believed to be both opportune and pertinent.

OD1S3 A strategy to approach 'welfare' assessment in cephalopods: the case of the common octopus (Octopus vulgaris)

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The main aim of the COST Action FA1301 CephsInAction Working Group 4 is to provide a collaborative framework for increasing knowledge on cephalopod welfare. One of the main outcomes of this framework is the attempt to develop a model for overall assessment of the welfare of octopuses in captivity. This model is proposed according to the principles of semantic modelling, in which welfare is defined as the quality of life as perceived by the animals themselves (Bracke et al., 1999ab).
We have performed a systematic literature review and collected more than 400 "statements" from scientific papers, using the criterion that the statements are somehow relevant to assess or describe the welfare of Common octopus. Based on these statements we first compiled a provisional list of 15 welfare needs: behaviour control, body care, exploration, feeding, health, kinesis, nutrition, osmotic balance, protection, respiration, rest, safety, sexual behaviour, predictable social contact and thermal regulation). Next, we created a list of 11 observable and/or measurable

welfare indicators (WIs): water temperature, oxygen level, stocking density, salinity, appetite, mortality, size dispersion, autophagy, cannibalism, latency of attack and skin lesions). The WIs were selected based on the criteria that each is indicative of at least one of the welfare needs, and that they can be divided into mutually exclusive levels from 'good' to 'bad' welfare. As an example, the temperature welfare indicator was divided into five levels, ranked from best to worst welfare as follows: (1) 17.5-20°C, (2) 15-17.5 °C or 20-22 °C, (3) 12-15 or 22-25 °C, (4) 10-12 °C or 25-28 °C, (5) 28 °C. To combine the WIs into an overall model, we used the statements and the weighting categories defined for semantic modelling by Bracke et al. (2002) to calculate weighting scores and generate a calculation rule for an overall welfare index (OWI). For instance, for the temperature WI we knew from the statements that level 1 gives increased positive performance (+3), while Level 4 gives significant reduced survival (-5) and probably illness (-5). The sum of the positive weighting categories subtracted by the sum of the negative gives a weighting factor of 13. Level 5 has very high probability of mortality and is therefore a knockout level. Knockout levels are levels considered to represent detrimental welfare and are by default leading to an OWI of 0. If no knockout levels apply, the WIs are aggregated into a scale from worst 0 to best 100, where the weighting factor of each welfare indicator determine their impact on the OWI. The advantages of this approach are that it gives a science based and objective assessment of overall animal welfare, it reduces multivariate data into a single index that can be used to compare the welfare of different populations, an overall index is also more robust to noise than individual WIs.

OD1S4 Experiments with Cephalopods: from best practice and protocols to 'procedures'

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Cephalopods are considered among the most 'advanced' invertebrates with highly differentiated brains, a sophisticated set of sensory organs and highly complex behavioural repertoires. They have been used for diverse scientific purposes for over 100 years providing the ground for studies that are at the foundation of modern neuroscience and behavioural biology. Here we review the use of cephalopods as laboratory animals, and the challenges offered by Directive 2010/63/EU to scientific community.
Rationale and Approach Based on empirical and factual evidences, the recognition of their 'sentience' and capacity to experience pain, suffering, distress and lasting harm, Directive 2010/63/EU (Article 1, 3b) on the 'protection of animals for scientific purposes' included this taxon, counting about 700 species, in the list of 'regulated' species, the sole representatives among invertebrates1.Over more than 15 decades of research made large use of different experimental approaches and paradigms to study cephalopods biology, physiology, nervous system, ecology and behaviour in order to understand the basis of their extreme adaptability and plasticity. We analysed scientific literature and experimental techniques ("procedures") and provided a temptative checklist based on the assumptions in compliance with the principles stated in Directive 2010/63/EU and animals well-being. Experiments that may cause pain, suffering, distress and lasting harm are also reviewed and cases discussed from the three most frequently species of cephalopods, i.e. the squid and the common cuttlefish and octopus. Cases will be discussed such as physiology, nociception, regeneration, behaviour and its development, the search of neural correlates. The use of anaesthesia in procedures1,2 as an approach to limit suffering and distress in animals will be also considered. Discussion and take-home message Based on examples we will propose different ways to consider cephalopods welfare in laboratory context, and will emphasize issues that require further efforts to refine 'procedures' and facilitating them fitting with the principles stated in Directive 2010/63/EU3.Our review and critical analysis of past and current best-practice are based on the recent networking efforts made by the COST Action FA1301 – CephsInAction. This is founded on principles of 'growing ideas through networks', and is aimed to foster the growth of the community; thus with the ultimate goal to fill the gaps in knowledge in cephalopod biology, helping crossing the bridge between best-practice and welfare-oriented approach of procedures in cephalopod experiments.

OD1S5 Developing training opportunities for cephalopods: the CephsInAction experience

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Cephalopods have been included in the Directive 2010/63/EU as the sole representative among invertebrates. A requirement is that staff taking care of animals and researchers should be adequately educated and competent. Currently, there is no Laboratory Animal Science education program including cephalopods. One of main goal of the COST ACTION FA1301 CephsInAction is to train people at different levels of expertise in the care, welfare and use of cephalopods for research, training and education.
CONTEXT AND METHODOLOGY: Two different approaches are followed to develop training programs that provide the basis for the acquisition of knowledge for the use of cephalopods as Laboratory Animals. The first approach is to include seminars or dedicated sessions with focus on cephalopods in more "general" courses programs. The other is to develop ad-hoc training schools, authorized by National Competent Authorities, have been carried out considering core and function-specific modules described in the 'Working document on the development of a common education and training framework under the Directive 2010/63/EU'. RESULTS: STATE OF THE ART: We review the currently available training programs for both 'experiences' in Europe and discuss cases and outlines of the topics delivered in different courses. Formal assessment of knowledge and skills is carried out through written and practical examinations. The various courses developed from 2014 in EU that include cephalopods in their program are presented. In addition, Cephalopods Biology and Care (CBC) Training Program have been developed under the aegis of the COST Action FA1301 with a total of more than 30 students of different nationalities (more than 10 countries, including Japan) involving lecturers and tutors from 7 countries. The courses developed by this experience are also presented and outcomes discussed. DISCUSSION AND CONCLUSIONS: CephsInAction educational programme started addressing main topics of cephalopods care and procedures to young researchers, sharing information related to the Guidelines for care and welfare of Cephalopods in research and the application of the Directive 2010/63/EU in the cephalopod community. One further goal of the CephsInAction is to provide training schools for fishermen to acquire competence for collecting and treating live cephalopods in the sake of their well-being, and for designated veterinarians. The field of health management in cephalopods still requires guidelines and information, but the whole community would benefit from widening this knowledge. A comparison of the two approaches is discussed and possible developments and cooperation are aimed.

OD2S1 How poor nutrition or feeding practice impact aquatic animals health

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Vetofish

Nutrition is part of health management, but often reduced to nutritional needs.
Nutritional diseases may be a predisposing cause of more obvious infectious disease. They are often investigated when all the other probable causes can be rule out, and as a result overlooked. A good food can rapidly be compromised depending on the way it is stored, produced or processed. The way and the frequency of feed delivery may also be deleterious. They have to be adjusted to the species physiology as well to abiotic factors. Through a couple of different and simple clinical cases we will present the most frequent nutritional pathologies noticed in research facilities and will broach the possible causes and how they can be avoided.

OD2S2 How to manage the water quality

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Laboratory aquatic units aim at an easy access to animals and they do not usually benefit from an advanced aquaculture technology or water quality control system. Moreover, assessing water quality should be a feasible method which can be carried out quickly on a day-to-day basis.
First we will explain how to easily monitor the main water parameters:

- Why 2 point calibration for pH is necessary
- TAN Vs NH3 – never measure NH3 without measuring pH

Then we will follow the transformation of NH3 through the Nitrogen cycle. Some bacteria (Nitrosomonas) recycle NH3 into NO2. Then Nitrobacter recycle NO2 into NO3. This will lead directly to a presentation of a seeding system that can be bio-secure and developed in the lab; it allows to seed the system in 2 weeks. From this practical example, we will discuss the variety of water test kits and how to choose according to the species requirements. Finally we will compare various guidelines on water quality for zebrafish and Xenopus spp and assess if they are met or not in common systems. This will trigger some questions on the suitability of the guidelines and/or of the systems regarding parameters like hardness, alkalinity and nitrate.

OD2S3 Health monitoring in fish and amphibians

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We develop a test panel in close collaboration to enable diagnostic testing of pathogens in zebrafish. An overview of the pathogens and diagnostic testing methods used to detect the pathogens will be presented.
Zebrafish and amphibians are frequently used as animal models in biochemical research. Like in rodent facilities, aquatic facilities may harbour infections, which can affect animal health. In addition, they also may influence the results of the investigation preformed with the infected animals or can be zoonotic forming a health risk for animal caretakers and researchers. It is therefore important to screen aquatic facilities to gain knowledge on these issues. Years ago we started to develop a test panel in close collaboration with zebrafish facilities to enable diagnostic testing of pathogens in zebrafish. An overview of diagnostic testing methods, sampling and frequently found infections in zebrafish facilities and a brief discussion if these methods and knowledge may also be used for health monitoring of amphibians will be presented.

OD3M1 Quality Control of Diets. Testing of nutrients and contaminants for GLP and other studies: past, present and future

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In the 1970s, Good Laboratory Practice (GLP) regulations included a need to measure concentrations of dietary contaminants that might affect regulatory studies. However, there was no clear statement of the specific contaminants, nor a need to measure nutrient levels. Most of the current panel of contaminants typically measured date back to the 1980s, and are in need of review. There is also a need for standardisation across Europe in contaminants tested and maximum limits in diet.
The dietary contaminants originally analysed by individual manufacturers to meet GLP requirements were usually those considered nationally to be of most significance, and this still results in variation between countries in the dietary contaminant routinely tested today. Individual companies and industry bodies such as BARQA and GV-SOLAS have also set limits to the amounts of contaminants in diet that also vary, though they must also comply in Europe with the directive 2002/32/EC. There have been few changes in the lists of contaminant typically tested since the early 1980s, and some of the original pesticides of concern are still included in routine testing even though rarely seen and largely absent from the environment in North America and Western Europe. Even the most recent legislation from the EU (2002/32/EC) still puts heavy emphasis on older pesticide residues rather than more modern ones. Nutrients are usually included in testing, and those included are largely at the discretion of the manufacturer, customers, and industry bodies but with regard to existing legal regulations such as EC/767/2009 that covers the nutrient declarations and the analytical tolerances for those declarations. In the FELASA Working Group on rodent diet and nutrition, we are re-examining the panel of contaminants for which testing is appropriate to attempt to standardise testing programs across the European Union. We are reviewing data on current contaminant levels in ingredients commonly used in rodent diets and determining which of the newer generation pesticides should be considered for routine testing, despite being of low toxicity. We are fortunate in gaining the support of most of the European laboratory animal diet manufacturers to identify which of the traditional contaminants still pose a potential problem and the typical and maximum levels one might expect to see in rodent diets. We are also reviewing whether the testing programme should include other potential contaminants and non-nutrients that might affect the outcome of the study, though we need to be conscious of the technical feasibility and the cost of any additional testing. We are also looking at the feasibility of standard tolerances around declared nutrient levels and the extent to which they will comply with EU standards, which are not always appropriate for laboratory animal diets. These issues and some of the difficulties in testing for nutrients and contaminants will be discussed.

OD3M2 Control diet in trial: comparison with standard or customized diets?

Martel, Dominique, Presenting author
SAFE

The ability to replicate research is fundamental for good science. Thus, Research team must control all variables except those being studied. To meet this requirement, the microbiologic and genetic characterization of laboratory animals has become increasingly well-defined over the years. Diet is an important environmental factor that affects reproduction, growth, disease, and response to experimental manipulation in laboratory animals. Hence, the choice of Control diet is essential.
In GLP and preclinical trials, new compounds are tested, thus standard diet is mainly required. In this case of drug development and toxicology assessment, the diet must feed the colony- group in order to be maintained in good health and conditions, and with the target to have low variables from diet. Whilst for Fundamental Research purposes, disease modelling, diet is a main route to lead the trial. Depending on their goals and models, Researchers can perform experiment with purified or standard diets. Over this presentation, we will focus on:· definition of standard vs purified diet: ingredients sourcing, design of formula, · main features of purified and standard diet: nutrients level and profile, specific nutritional value attached formula design, · specific requirements as deficient diets (Sodium, vitamins, iron) and enriched diets (High fat diets, high sucrose, high fibre), MCD, · hazards and impact attached to standard and purified diets: contaminants and specific micro nutrients and behavioural measurements. Finally, a discussion related to the choice of control diet pertaining to this trial: which comparison must be performed, a control standard or a control purified diet. By this choice, results and interpretation can be changed.

OD3M3 Minimizing the risk of introducing parvovirus through food by irradiation or autoclavation

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The decision to administer a non-sterilized diet food to mice resulted in an outbreak of a mouse parvovirus infection in a laboratory animal facility in the Netherlands. The temperatures that are reached during the processing of the food pellets are apparently not high enough to kill the parvovirus. Temperatures or irradiation doses required to inactivate the virus have been investigated.
Parvoviruses are resistant to temperatures up to 80oC. This is also approximately the maximum temperature that is achieved during the preparation of the food grain pellets used for laboratory animals. Sterilization or autoclavation could have prevented the infection. The question remains which irradiation dose is required to inactivate parvovirus as this is probably the only viral pathogen that can resist a temperature of 80°C.This presentation will give insight into the inactivation of parvoviruses and consequences for interpretation of diagnostic test results.

OD4S1 From the bush to the bench: the annual fish Nothobranchius furzeri provides novel insights in the biology of aging

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Annual Nothobranchius fishes are small teleosts that inhabit temporary water bodies in Eastern Africa subject to annual desiccation due to the alternation of the monsoon seasons. Their increasing popularity stems from the extremely short lifespan that is the result of their specific life-history adaptations and is retained under laboratory conditions. Nothobranchius furzeri, the most popular laboratory species, is the vertebrate species with the shortest lifespan recorded in captivity.
It was shown that their short lifespan entails rapid age-dependent functional decline and expression of cellular and molecular changes comparable to those observed in other vertebrates, including humans such as cognitive decline, locomotor impairments, mithochondrial dysfunctions, telomere shortening, iron accumulation, oxidative stress and neoplasias. Further, the genome-wide regulation patterns of gene expression observed during aging overlap with those observed in humans. These observations make N. furzeri a unique model system to investigate the effects of experimental interventions on aging and longevity. The availability of a highly-contiguous genome assembly and the development of transgenesis in this species make it possible to test the effects of genetic manipulations on the genome whereas the establishment of transgenic lines is facilitated by their very rapid generation time, which can be as short as one month. This model system allowed in recent times to reveal novel hitherto unknown aspects of vertebrate aging:

1. aging-related genes tend to cluster into specific genomic regions,
2. gene expression profiles observed during embryonic development and aging partially overlaps,
3. genes responsible coding for complex I of the respiratory chain and genes responsible for expression of mitochondrially-encoded proteins are under positive selection in Nothobranchius fishes
4. expression of the same genes is negatively correlated with individual lifespan in longitudinal analysis of gene expression
5. partial pharmacological inhibition of complex I induces life-extension and rejuvenation of the transcriptome
6. microRNA mir-29 controls a compensatory response to limit neuronal iron accumulation during adult life and aging.

OD4S2 Medaka fish as a model system in biomedical research

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Medaka Oryzias latipes represents a precious model for biomedical research. The increase in the number of genomic resources available, combined with the detailed linkage map and the high number of inbred strains that have been established, make medaka a suitable model for the study of gene function. In biomedical research, medaka is used in large screens of drugs thanks to its simple maintenance and its high rate of proliferation that allows the analysis of the effects in hundreds of embryos per time.
Medaka fish, Oryzias latipes, is a teleost fish of the family Adrianichthyidae, native to Taiwan, Korea, China and Japan. Its natural environment is characterized by temperate zones and thanks to this medaka can tolerate a wide range of temperatures and salinities. The life span of medaka in the laboratory conditions with 14 hours light and 10 dark at 25-28°C is almost 12 months. Males and females can easily be distinguished by a clearly dimorphic dorsal fin. Medaka is an oviparous fish, usually 30 to 50 eggs per day could be spawned every day and reproductively active female can be easily visualized and propagated by the eggs that are connected to female body through filaments. The eggs and the embryos are transparent and the hatching period is reached after 7-8 days at 28°C.One of the first reported used of medaka in scientific research is dated 1913 and focused on the Mendelian inheritance in vertebrates. In 1986 medaka was the first species in which stable transgenesis was established and already in 1991 it was used to create multi locus tester strains by Shima et all. In more recent years medaka has been one of the first vertebrate models used to study the life cycle aboard the International Space Station. In last decades, medaka has been widely used in developmental biology to characterize the major events of eye development, and for the study of sex determination. Despite this important role in developmental biology, the main characteristic of medaka fish is its suitability in biomedical research. Rapid development, economic husbandry, high fecundity and rapid ex utero development, together with the permeability of the skin for small molecules are just few of the several advantages of medaka, over traditional mammalian model systems. Given these features medaka as organism model can be exploited in drug development process, drug target identification, estimation of drugs and environment toxicity. Moreover thanks to the genomic resources and the several approaches routinely used to analyse gene function and induce mutagenesis, medaka represents a powerful genetic model system. This characteristic can be exploited in order to create mutant medaka fish with defective phenotype that can fully photocopy the one of human disorders. Therefore transgenic medaka fish offers a reliable platform for high-throughput screening of drugs and for the identification of the molecular mechanisms responsible for disease pathogenesis in vivo.

OD4S3 Husbandry, welfare and health aspects of the shortest-lived vertebrate, the annual teleost fish Nothobranchius, a newly established model organism in basic and biomedical research

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The biological mechanisms underlying ageing are entering the spotlight of research, due to the demographic changes and increase of human longevity with associated age-dependent morbidity. In this context, the teleost genus Nothobranchius, as short-lived vertebrate model for ageing research, is gaining popularity over the mouse as the model par excellence. It is therefore necessary to define requirements of husbandry, welfare and health aspects in laboratory conditions for this species.
The annual teleost of the genus Nothobranchius is the vertebrate with the shortest lifespan recorded in laboratory conditions. In last decades, its biology has been intensively investigated and the interest of the scientific community is constantly increasing. This is due to the rapid decline of cellular functions, which is age-dependent and follows the same molecular rules of higher vertebrates (Genade et al.,2005). The peculiar biology highlights this fish as a model in many disciplines. Several metabolic and degenerative diseases as well as tumour development (Di Cicco et al., 2011) are described, which are interesting for pathologists and immunologists. Also, the life-cycle of this fish represents a fascinating subject for embryology and evolutionary studies, thanks to its peculiar embryonic developmental arrest, that may occur at three different diapausal stages. The sequencing of Nothobranchius genome (Reichwald et al., 2015) and the establishment of transgenic lines using different tools, including CRISPR/CAS9, makes it suited for multidisciplinary investigations. Nevertheless, Nothobranchius facilities are widely spread, designed guide lines about health, husbandry, monitoring and management do not exist yet. It has been reported that discrepancies in rearing and maintenance could lead to apparently contradictory experimental results. In fact, several factors could have strong influence on the experiment outcome, such as food composition, dietary regime and all the parameters involved in metabolic rate, oxidative stress and pathologic condition. Starting from the biological necessities of Nothobranchius and its welfare, we analyse optimal temperature, type and frequency of feeding, photoperiod, population density, sex ratio and reproduction, to give the basis for standardise the protocols for maintaining colonies. Particular attention is also given to the management of health monitoring, diagnosis and treatment of pathologies, which can affect animals in facility and could also represent a zoonotic risk. Our aim is to establish a guide to promote the extension of Nothobranchius model based on adequate, coherence and safety principles.

OD4S4 Housing and caring for three spined sticklebacks (Gasterosteus aculeatus) in a research aquarium facility

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The three-spined stickleback has become a major biological model over recent years, yet unlike other fish models there is currently no standardised accepted protocol for housing and care of the species in research aquaria. Individual facilities typically adapt existing aquarium resources to accommodate the new species, and this may lead to suboptimal housing.
Sticklebacks have long been used as models for behavioural research, but in more recent years their utility as model species in biological sciences has increased dramatically since the publication of their genome and associated tools, and they are now routinely used in studies of genetics, ecotoxicology, parasitology and evolutionary biology. They are particularly commonly used as models in studies of environmental change, since their suitability to laboratory studies is matched by a detailed knowledge and understanding of their natural history and ecology in natural populations. Here, we present findings of a survey of housing conditions used in stickleback aquaria worldwide, relate these to the natural ecology of the fish, and provide an insight into the successful housing and care regimes developed and used by the fish facility at the University of Leicester, UK. The following aspects of sticklebacks care in laboratory aquaria will be addressed.
Diet – We will outline our feeding protocols, from first feeding through to feeding of adult fish.
Environment conditions and enrichment – We examine water quality parameters, lighting and temperature regimes, tank sizes and stocking densities, as well as looking at the importance of environmental enrichments.

Breeding and embryo care – We will introduce both natural spawning and IVF approaches to embryo generation, and how to achieve the maximum clutch yields and outline our practices for egg and embryo maintenance.
Health and disease – We introduce the common parasites and diseases that can occur in a stickleback facility and how to treat them effectively.
Transportation – Finally we explain our protocols for transferring wild-caught sticklebacks to the facility and the procedures we follow to maximise healthy and minimise stress during transportation and after arrival.

OD5M1 Revised Recommendations for the Health Monitoring of Rodent and Rabbit Colonies - FELASA Working Group (2014)

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CNR

FELASA working group on revision of guidelines for health monitoring of rodents and rabbits. Laboratory Animals 2014, Vol. 48 (3) 178–192
Phenotyping may influence the suitability of laboratory animal models in research. The occurrence of infectious agents in breeding or experimental laboratory animal facilities highlights the need to consider the animals' microbiological quality since it directly influences welfare, experimental variability and scientific research projects. The use of animals of known biological pattern is important in ensuring reproducibility of experimental results.
The microbiological quality of experimental animals can critically influence animal welfare, ethics and the validity of reproducibility of research data. It is therefore important for breeding and experimental facilities to establish a laboratory animal health monitoring (HM) programme as an integrated part of the quality assurance system. FELASA has published recommendations for the HM of rodent and rabbit colonies in breeding and experimental units (Nicklas et al Laboratory Animals, 2002), with the intention of harmonizing HM programmes. These recommendations need to be adapted periodically to meet current developments in laboratory animal medicine. In the meantime, sciences evolved, new methodologies were investigated and new organisms are considered to be relevant for health screening. New assessments were made if certain organisms have to be regarded as pathogenic or not. These recommendations are aimed at all breeders and users of laboratory mice, rats, Syrian hamsters, guinea pigs and rabbits as well as diagnostic laboratories. They describe essential aspects of HM, such as the choice of agents, selection of animals and tissues for testing, frequency of sampling, commonly used test methods, interpretation of results and HM reporting. Compared with previous recommendations, more emphasis is put on the role of a person with sufficient understanding of the principles of HM, opportunistic agents, the use of sentinel animals (particularly under conditions of cage-level containment) and the interpretation and reporting of HM results. Relevant agents, testing frequencies and literature references are updated. Supplementary information on specific agents and the number of animals to be monitored and an example of a HM programme description is provided in the appendices.

OD5M2 Contemporary approaches to optimal rodent health monitoring

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IVCs are a great tool for protecting animals and operators however their use has caused great limitation to the efficacy of health monitoring practices. The use of dirty bedding sentinels have been the gold standard for more than a decade but with a lot of limitation due to many variables (prevalence, route of transmission, age, strain of the sentinel etc). Modern technologies might allow different approaches and look promising. However all that glitters might not be gold.
Micro-isolation cage systems (mainly IVCs and FTCs) are widely used nowadays, with the aim of protecting animals and operators. Health monitoring of these units has always been problematic because each cage is in reality an independent microbiological unit. Traditionally these units are monitored with the use of a variable number of immunocompetent sentinels that have been exposed to dirty bedding, tested via serology, bacteriology and parasitology. This procedure relies upon the transmission (not always efficient) of agents in addition to other uncontrollable variables such as prevalence of disease; dose of agents that are shed by resident animals; frequency and amount of bedding transferred in addition to the susceptibility and receptivity of the sentinels. The hypothesis is that modern technologies allow sampling points other than sentinels to make the screening more sensitive. We analysed and compared results obtained in different sampling points (sentinels, principal animals, environment) and different methodologies (mainly serology, PCR and parasitology) for agents believed to transmit inefficiently to sentinels via dirty bedding. Apparently a better health monitoring is obtainable by combining tests conducted on different sampling point depending on the agent of interest.

OD5M3 Exhaust Air Dust Monitoring is Superior to Soiled Bedding Sentinels for Detection of Pasteurella pneumotropica in Individually Ventilated Cage Systems

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Reliable detection of unwanted organisms is essential for meaningful health monitoring in experimental animal facilities. Since transmission of many pathogens such as Pasteurella pneumotropica (P.p.) via soiled-bedding to sentinel mice is insufficient [1, 2], infectious agents often go undetected in IVC systems. In this study, we investigated whether analysis of exhaust air dust (EAD) samples is superior to conventional SBS monitoring for detection of P.p. infections.
In an experimental IVC rack with known P.p.-prevalence, weekly EAD sampling was compared to the classical SBS method over 6 periods of three months. EAD samples were analysed using a specific and sensitive real-time PCR assay [3]. An experiment using a decreasing quantity of P.p.-positive mice was performed to investigate the minimal prevalence detectable by EAD PCR. In six rounds of testing over 3 month with a prevalence of 5 infected mice in each of 7 cages in a rack of 63 cages, EAD PCR detected P.p. each week, whereas the SBS method failed to detect P.p. in all instances. Although substantial amounts of contaminated faeces were transferred to the sentinel cages, SBS had not become infected. The minimal prevalence of P.p.-infected mice required to obtain a reliable result by EAD PCR was determined to be 1/63 cages. Reproducible detection of P.p. was achieved already after one week. As demonstrated, SBS monitoring is inappropriate for P.p. detection. EAD PCR enables efficient monitoring of experimental animal facilities with reasonable sample numbers. Analysis of EAD samples by real-time PCR provides a sensitive, simple, and low-cost approach for P.p. identification at medium and low prevalence and has great potential to become a useful tool for primary health monitoring surveillance of rodent populations.

OD5M4 High-Resolution Melting Curve Analysis for Identification of Pasteurellaceae Species in Experimental Animal Facilities

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Pasteurellaceae are among the most prevalent unwanted organisms isolated from mice housed in experimental animal facilities [1]. Reliable detection and differentiation is essential for proper health monitoring. Yet, standard procedures for detection and identification of Pasteurellaceae in mouse colonies are either unspecific, or expensive or time-consuming.
We have combined a real-time PCR assay [2] amplifying a variable region in the 16S rRNA sequence with high-resolution melting curve analysis (HRM) to identify and differentiate among commonly isolated Pasteurellaceae species. Double-stranded DNA-binding dyes are used in conjunction with precise temperature ramp control and advanced data capture capabilities to detect small differences in PCR melting curves of different species and strains based on sequence variations [3]. We used a set of six reference strains (Pasteurella pneumotropica biotype "Jawetz" and "Heyl", Actinobacillus muris, and Haemophilus influenzaemurium) for assay development. For evaluation, 25 unknown Pasteurellaceae isolates obtained from an external diagnostic laboratory were analysed. The melting temperatures of the reference strains were sufficiently different due to DNA sequence variations. The melting curves of each of the 25 isolates matched one of the reference strains. HRM identification of 23 isolates was consistent with partial 16S rRNA sequencing. Species-level identification by HRM of two H. influenzaemurium isolates was correct, but sequencing displayed two opposite and adjacent single-nucleotide modifications (A/G and G/A vs. G/A and A/G) compared to the related reference strain, which did not affect the melting temperature. The real-time PCR/HRM method provides a sensitive, rapid, and closed-tube approach for Pasteurellaceae species identification. This simple and low-cost method has great potential to become a useful tool for research and diagnostic laboratories for health monitoring of laboratory mice which can be easily adapted to other pathogenic bacteria.

OD6M1 Monitoring health of zebrafish

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Diagnostic laboratories have recently developed PCR panels to allow identification of pathogens in zebrafish. These tests can be performed on fish or environmental samples. But do they cover only relevant pathogens to screen and are they exhaustive?
We will discuss why monitoring the health of zebrafish is necessary. Then we will list the main pathogens to consider according to their pathogenicity and prevalence. To detect efficiently the pathogens of interest and to reduce the number of tested animals, some environmental samples can be used and setting sentinels is an option. We will describe the various environmental samples that can be performed. Similarly, setting sentinels requires some adaptation to the system and we will see how to avoid some deadly mistakes. Finally, PCR are specific to defined pathogens whereas some surveillance is still necessary in zebrafish facilities when the health status control is not optimal. Therefore some more general health monitoring should be in place in the facility in order to detect unexpected pathologies (e.g. histo-pathology) and to control morbidity and mortality.

OD6M2 Comprehensive analysis of a Zebrafish Health Program allows characterization of the most prevalent signs, lesions and pathogens in a research facility and assessment of quarantine policy efficacy

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In the past two decades, zebrafish (Danio rerio) -based research has contributed to significant scientific advances (1). Still, husbandry and health programs did not evolve at the same pace, despite being essential to animal welfare, to robust experimental data and to permit animal exchanges across facilities. A Health Program was initiated in 2011 and has been gradually updated since then. The main objectives are to prevent, monitor and control pathogen and disease dissemination.
Materials and Methods The Zebrafish Health Program consists on a set of protocols and policies that include quarantine and animal importation, embryo surface disinfection, daily animal observation, routine health screens of sentinels and non-routine analysis of healthy and sick/moribund animals (2). Health screens comprise necropsy, histology and bacteriological culture of animals and water. Results The combination of the results of necropsies, histology and bacteriological cultures allowed prevalence determination of signs, lesions and pathogens (3) in three sample groups: sentinel animals, randomly picked animals (non-routine) and sick/moribund fish. The most prevalent pathogens found are Pseudoloma neurophilia (3.6% in sentinels; 6.4% in non-routine; 0.5% in sick/moribund) and Acid Fast Bacteria (AFB, presumably Mycobacteria spp.; 1.8% in sentinels; 5.5% in non-routine; 3.8% in sick/moribund). In the sick/moribund group, the most frequent lesions are: aerocystitis (swim bladder lesion; 24.6%); cutaneous ulcers (23.6%), peritonitis (coelomic cavity lesion 17.7%), branchitis (gill lesion; 16,9%) and neoplasia (9,8%).The comparison of pathogen prevalence across different zebrafish holding rooms indicates a significant reduction of Pseudoloma neurophilia from the Quarantine (17.6%) to the Central Facility (3.6%). AFB prevalence also shows a tendency of reduction, from 6.1% to 1.8%. The analysis of the most prevalent lesions and pathogens across animal age shows that AFB, Pseudoloma neurophilia, aerocystitis, cutaneous ulcers, neoplasia and peritonitis increase prevalence with aging. Discussion and Conclusion This analysis enabled a comprehensive description not only of pathogen prevalence but also of lesions and disease frequency of resident animals. The establishment of a quarantine program revealed to be effective in the reduction of Pseudoloma neurophilia frequency in the Central Aquaria Facility. We also report age-associated lesions and pathogens, supporting the need of effective animal age policies in research settings. Finally, characterization of the colony health status based on this program unravels epidemiological data, whose interpretation needs to be put in context with that of other facilities, therefore awaiting more data availability on this subject.

OD6M3 Environmental and ante mortem samples for zebrafish health monitoring and quarantine

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Zebrafish colony health monitoring often includes testing sentinel and/or colony fish. The efficiency of transmission to sentinels varies by pathogen, and direct testing of colony fish often detects high prevalence pathogens, but requires a very large sample size to detect uncommon agents.[1] PCR allows evaluation of a wide variety of sample types. Environmental samples could be a useful adjunct to sentinels, and ante mortem samples could expedite quarantine without euthanasia of valuable fish.
Materials and Methods. We compared the utility of environmental and ante mortem samples to sampling adult zebrafish from two populations that were enzootically infected with multiple zebrafish pathogens. Mycobacterium chelonae, M. fortuitum, M. peregrinum, and P. neurophilia were present in both populations, whereas M. haemophilum was present only in population B and Pseudocapillaria tomentosa was present only in population A. We compared real-time PCR detection of multiple pathogens in multiple sample types, including zebrafish, tank detritus (sediment), 0.2 um filter membranes following filtration of 1 L, 500 mL, and 150 mL water samples, as well as pooled embryos and pooled feces that were collected overnight from groups of six zebrafish from each population. Both populations were restocked, cohoused for one month, and the experiment was repeated under lower prevalence conditions. Results. Diagnostic sensitivity varied according to sample type and pathogen. Environmental sampling was significantly more sensitive than sampling zebrafish for detecting Mycobacterium spp. in both populations evaluated. All detritus samples from population A tested positive for P. tomentosa, whereas filter membranes displayed a trend toward improved detection when higher volumes of water were filtered. In contrast, filter membranes were more effective than detritus samples for detection of P. neurophilia in both populations when prevalence was high. Mycobacterium spp. and P. tomentosa were often detected in pooled fecal samples, whereas detection of P. neurophilia was poor. Pooled embryos performed poorly as a diagnostic sample for all pathogens tested. Discussion and Conclusions. This study supports evaluation of multiple sample types, including both fish and environmental samples, to gain a better understanding of zebrafish colony health. Excellent detection of mycobacteria in environmental samples reflects their dual lifestyle as facultative pathogens that proliferate in the environment. In contrast, P. tomentosa and P. neurophilia are obligate parasites that persist in the environment as eggs or spores, but only reproduce within the host. Pooled fecal samples are valuable ante mortem samples for mycobacteria and P. tomentosa, although sensitivity for P. neurophilia was relatively low. Pooled embryos were not a sensitive sample type, suggesting that negative results from embryo samples may not reflect the health status of the parents.

OD6M4 RESAMA: an aquatic research facility health monitoring network

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Fish and Amphibians aquatic model-animals are important tools for modern biology. There external development make them suitable for a great variety of studies. Health management of these species is, however, poorly understood and certainly a few steps behind what is done for mice. The relative lack of knowledge of pathological mechanisms in the aquatic non-mammalian vertebrates and a shortage of trained veterinary specialists are the identified causes of this situation.
The French aquatic animals' user scientific community aware of this drawback decided to address this problem by structuring an aquatic rearing facility health monitoring dedicated network RÉSAMA (RÉseau de Surveillance sanitaire des Animaux Model Aquatiques). The network is coordinated by two public resource centres AMAGEN (fish species) and the CRB Xenope (amphibians) together with the financial support of the TEFOR infrastructure. Now federating over 60 French research rearing facilities, RÉSAMA has engaged concrete actions to promote health monitoring in aquatic research facilities, starting with a nationwide survey of health and husbandry practises in research facilities. Teams composed at least of one veterinarian and one zootechnician specialised in the aquatic species assess the health and the rearing practise of research facilities all around France. The aim is to visit all the facilities composing the network and to draw up guidelines regarding both health management and husbandry practices. We believe these actions will help to improve health management in aquatic facilities. They will allow to build a guideline of best practices based upon rational evidences and will generally promote dialogue over these questions. This initiative will also increase awareness toward health management amongst veterinarians and scientists, ease and secure exchanges between research institutes and improve efficiency and repeatability of biological experiments. Here, we will describe the results from the first 23 visits realised so far and discuss some of the conclusion to be drawn from these first analysis as well as the future of the project.

OD7W1 Implementation of a Gnoto/Axenic Facility: a case report from the Instituto Gulbenkian de Ciência

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The field of Gnotobiology has grown exponentially over the last few years. The demand for germ-free animals available for experiments exposed the need for expansion in Axenic Facilities. Here we present a historical report of the implementation of the Gnoto/Axenic Facility of the Instituto Gulbenkian de Ciência, with emphasis in equipment, quality control, human, financial and space resources.
The Germ-Free (GF)/Axenic Facility of the Instituto Gulbenkian de Ciência (IGC) started in 2005, with rigid walls Isolators, for axenization and maintenance of mutant and wild-type mouse strains of different genetic backgrounds. The facility is open to the International community through the EMMA/Infrafrontier consortium. In 2013, a gnotobiology facility was implemented for experiments with GF animals. For this, the IsoCage P system has been in use. Each cage is a micro-isolator, allowing multiple studies on the same rack, providing full bioexclusion for maximum animal protection, excluding cage-to-cage contamination. To validate our working system, standard operating procedures (SOPs) and GF sentinel were

implemented. These animals are manipulated exactly the same way experimental animals do, and their health status checked every month for quality control. Experimental animals are microbiologically tested in the beginning of the experiments and the respective controls at the end. The use of the IsoCage P system for gnotobiology experiments allowed freeing space in isolators and expanding the number of GF animals produced. From 2013 to 2015, 44 experiments were performed using axenic animals, registering a decrease of contaminations from 45% to 5-10%. Currently, the Gnoto/Axenic facility is running with seven isolators, two IsoCage P systems, breeding four different axenic strains, has a capacity of 400 deliverable animals and at least two EMMA/Infrafrontier research projects concluded per year. Axenization of new strains is performed by c-section rederivation and adoption by GF foster mothers, with high rate of success. All isolators follow a health monitoring program that consists of microbiology testing in house once a month and a FELASA annual panel every six months. Major concerns are to avoid contaminations and technical problems, such as isolator breakdown. In order to face such situations, strains are always housed in two isolators. In addition, one isolator is always kept as a backup for speed expansion of the affected colonies. Our Gnoto/Axenic facility has highly specialized and dedicated personnel. It is integrated in the Animal House Facility, which allows resources optimization such as equipment (sterilizers, washing machines), basic supplies (diet, bedding, beverage), human resources and general facility workflow (staff and animals circulation).

OD7W2 Gnotobiotic Unit at the Central Animal Facility and Institute for Laboratory Science of the Hannover Medical School

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The Institute for Laboratory Animal Science and Central Animal Facility of the Hannover Medical School (Ztm) has a long tradition of gnotobiotic work. Since unit establishment it supports scientists in germ free (GF) work and breeds different GF mouse and rat models. Interest in GF models inclined over the past decade with increased interest for the microbiome research. Therefore, gnotobiotic models were recognized as a valuable tool for studying complex host-microbiota interactions.
The German Research Foundation (DFG) has established in 2013 a Priority Program entitled "INTESTINAL MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation" (SPP1656) with the focus on molecular mechanisms underlying the functional interactions between microbiota and the intestine under normal, infectious and chronic inflammatory conditions. The Ztm participates in this program since the program started and serves as a core gnotobiotic unit for the scientist participating in the program. Since its establishment the gnotobiotic unit expanded in number of isolators and strains kept GF. Today it counts 34 Hannover type isolators. The main goal of the unit is to generate and maintain GF and gnotobiotic models. GF models are mainly re-derived by caesarian section method. However, the sterile embryo transfer method is also established. Furthermore, the unit performs gnotobiotic experiments in a collaborative manner and supports the researchers within the SPP1656 program as well as others by providing our technique, experience and knowledge. In more than 80% of unit available isolators, different mouse models are kept, including 7 wild type and 13 mutant strains. Additionally, our unit has two GF rat strains available. Furthermore, the unit has isolators housing mouse strains carrying specified bacterial flora such as Altered Schaedler Flora and Segmented Filamentous Bacteria. In 2015 the gnotobiotic unit successfully re-derived and established GF breeding for 7 different mouse models. The gnotobiotic facility of the Ztm successfully implemented its techniques and expertise in many collaboration projects mostly including research groups participating within the SPP1656. For the short term gnotobiotic experiments specifically designed Hannover gnotocages are in use, which can also be used for the sterile animal transport. Overall within last years´ Ztm gnotobiotic unit offered its expertise and techniques in gnotobiology field and accomplished Germany-wide but as well as worldwide collaboration with research groups. Our facility aims to further pursue this path of successful collaborations.

OD7W3 Maintaining and Monitoring the Microbiological Status of Gnotobiotic Rodents

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The microbiome of experimental animals can severely influence the outcome and quality of scientific data. Great efforts are therefore necessary to maintain and monitor a defined microbiological status in breeding and experimental facilities by establishing a health monitoring programme. In the last 10 years, Gnotobiotic animal models were recognized as a powerful tool for studying the host-microbiota relationship. A hallmark of the gnotobiotic status is the underdeveloped immune system of the animals and an increased risk for contamination through handling or technical failures. The maintenance and monitoring of these colonies have become a major challenge in establishing housing techniques and management as well as in developing health monitoring programmes. The health monitoring programme of the Institute for Laboratory Animal Science and Central Animal Facility of the Hannover Medical School (Ztm) has been for a long time established and reliable. Here we want to provide information on the housing and maintenance on gnotobiotic animals and make specific recommendations for sampling procedures, frequencies and test methods.

OD7W4 Use of Hermetically Sealed, HEPA-Filtered, Positive-Pressure Cages for Experiments with Gnotobiotic Mice

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Germ-free mice are often used to study the role of the gut microbiota in both health and disease. These mice are generally housed, bred, and maintained in flexible-film isolators to sustain their germ-free status. In experiments in which germ-free mice are colonized with a single bacterium or a mix of bacteria, each specific donor microbiota sample requires its own isolator to prevent introduction of unwanted microorganisms. The requirement to use a separate isolator for each different microbiota sample greatly increases the number of isolators needed to conduct colonization experiments especially when multiple donor samples are used. Therefore, we describe the use of hermetically sealed, HEPA-filtered, positive-pressure cages for short term maintenance of germ-free mice and for colonization experiments. Each cage in effect functions as an individual isolator. Using this caging system along with aseptic technique and a biological safety cabinet, we housed and maintained germ-free mice for 12 weeks. Additionally, a germ-free breeding pair was confirmed to remain germ-free through two subsequent litters, and both litters were likewise confirmed free of contaminating microorganisms for 4 weeks after weaning into a new cage. Lastly, we used this caging system to perform colonization experiments and showed that after transfer of faeces from SPF mice into germ-free mice, the microbiota stabilizes as early

as 2 weeks post transfer even though the recipient microbiota did not completely recapitulate the donor microbiota. These data indicate that use of hermetically sealed, HEPA-filtered, positive-pressure cages along with aseptic technique is a suitable alternative to isolators when conducting experiments with germ-free or gnotobiotic mice.

OD8M1 Lean, Green and Clean – Approaches to Vivarium Design that will save you operational cost and environmental impact

Zynda, Jeffrey, Presenting author
Perkins + Will

Legacy thinking regarding laboratory animal facility design propagates inefficient and ineffective design solutions from an energy use, carbon reduction, and operational efficiency perspectives. Using up-front planning approaches and techniques can result in efficient, effective and operational cost-reducing vivaria. This methodology will be shared with audience to avoid design issues on new and renovated facilities.
Labour and utility consumption are the two greatest operational cost-centers in a laboratory animal research facility. Often, during design the most emphasis is placed on the first cost of investment (facility). In other terms many designers, owners and architects are primarily focused on the facility, without sufficient consideration of the life-cycle impact of their design decisions. Decades old metrics and ideologies about automated technologies for wash, handling and process operations are still pervasive across the globe, leading to inefficient and costly operational budgets as well as unnecessary environmental impact. This unfairly shifts the predominant cost burden to the end-user or facility operator. As budgetary constraints continue to be a global limiting factor, institutional stewards should be aware that alternate up-front design process methodologies and planning approaches can yield operational savings for the lifespan of their animal facilities. This session will explore the benefits that "process oriented" design can have on facility efficiency, both spatially and operationally, as well as the long-term operational cost reduction. Emphasis on reduced environmental impact will frame this discussion. Techniques and methodologies to evaluate energy efficient equipment and facility design with be outlined. LEAN process to optimize flow and function will be also be shared. This presentation will brief the audience on significant areas (such as cage-wash and handling) where cost and energy consumption can be reduced and how they can engage their design teams in a dialog to prioritize these issues.

OD8M2 How Lean can support efficiency of Laboratory Animal facilities operations

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Lean production is a management philosophy derived from the Toyota Production System, which considers the expenditure of resources for any goal, other than the creation of value for the end customer, to be wasteful, and thus a target for elimination. Lean has been successfully applied to many activities of laboratory animal facilities from the design phase to the operation of animal care programs.
The authors manage a 600 m2 mouse facility housing around 6,000 ventilated cages. Over the years, several upgrades (ranging from the introduction of dirty side automation systems, to optimization of the logistics) were implemented in order to increase the efficiency of the operations. In order to further improve, the main animal care activities were re-evaluated and reorganized using a Lean approach approximately five years ago. The initial transformation was carried out with the support of a team of consultants and involved mainly cages and bottles processing, both in the animal rooms and in the washing area. Activities were balanced and new workflows defined together with new process layouts and time schedules. Lean was subsequently spread also to the areas not involved in the initial transformation, such as supplies management, daily cages and health checks, and breeding activities. The results of the application of Lean management were striking, both initially and over the five years of continuous implementation, and will be discussed together with the challenges encountered during the process.

OD8M3 Evaluation of the Toxicity and Potential Oncogenicity of Power Frequency (50/60 Hz) Magnetic Fields in Animal Models Commonly Used for Hazard Identification and Safety Assessment

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Power frequency [50/60 Hz] magnetic fields (MF) are produced by electrical equipment that includes appliances, building wiring, circuit panels, and overhead power lines. Because MF are ubiquitous and cannot be effectively shielded, public concern has emerged regarding possible health effects of MF. This presentation will review studies performed to identify and characterize possible health effects of MF exposure in laboratory animal models with demonstrated predictiveness for human responses.
Many well-designed and conducted studies have been performed to evaluate the toxicity and potential oncogenicity of power frequency [50/60 Hz] magnetic fields (MF) in laboratory animals. Integration of these experimental data with data from epidemiology studies can support a comprehensive assessment of the possible health hazards of human exposure to MF. The results of acute, subchronic, and chronic toxicity evaluations conducted using standard rodent models for safety assessment provide no evidence that exposure to MF induces significant systemic or organ-specific toxic effects. Evaluations of the developmental and reproductive toxicity of MF in models that are commonly used to identify reproductive hazards have been uniformly negative. Immunotoxicity bioassays of MF have generated both positive and negative results; however, positive findings have not been linked to any functional deficit in immunity. The results of chronic bioassays in rodents provide no evidence of MF oncogenicity in any organ system. Similarly, the results of multi-stage oncogenicity studies in organ-specific animal models for skin cancer, brain cancer, and lymphoid/hematopoietic neoplasia provide no evidence that MF act as either a co-carcinogen or tumour promoter in these sites. The results of studies in genetically engineered (transgenic and gene knockout) animals demonstrate no activity of MF as a promoter of hematopoietic neoplasia in sensitive subpopulations. The only experimental evidence of tumour promotion by MF is in the rat mammary gland; however, although enhancement of mammary carcinogenesis by MF exposure has been reported by two laboratories, attempts at independent replication of these results have failed. The experimental literature provides no consistent body of evidence to support the hypothesis that exposure to MF is a significant risk factor for human toxicity or neoplastic development in any site. No generally accepted biophysical or molecular mechanism for MF toxicity or oncogenicity has been identified, nor has any robust body of in vivo evidence linked MF exposure to alterations in any cancer-associated molecular biomarker. In view of the lack of any reproducible demonstration of MF toxicity or oncogenicity in any organ system in laboratory animals, it is concluded that the published experimental literature provides little support for the hypothesis that exposure to MF poses a significant risk to human or animal health.

OD8M4 Technology as aid to effectively address the stink about ammonia

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Despite the lack of standards for acceptable levels of ammonia in rodent housing, averting its effects can be accomplished through technologies which address the moisture before it can have an impact
In the vivarium ammonia is a simple molecule chemically, one nitrogen and three hydrogen atoms, but it has complicated and varied concerns in terms of both animal and personnel health. As part of metabolic processes, nitrogen is rid as the waste product urea in most terrestrial animals. In the presence of anaerobic bacteria and moisture, the urea is converted to ammonia, which as a heavy gas remains present within the animal's caging environment. If not addressed in a timely manner, which typically is a hygienic task of some kind with a certain frequency, adverse effects can arise. Such effects depend upon the strain of animals, their health, enclosure density, the diet, the type of bedding, type of housing and other factors. In murine studies, irritation and pathology of the respiratory tract have been documented. For vivarium personnel, ammonia is an irritant to the eyes, skin, and respiratory systems. Mice seem to be much more tolerant of high ammonia concentrations (~100 ppm), whereas human exposure is limited by US-OSHA to 50 ppm during the work day. While ammonia is regarded as relatively toxic, there is little agreement within the lab animal community regarding safe levels for mice. The problem is that few studies have been done to better understand the implications. Another aspect is the inability to detect (sense) and measure ammonia accurately with minimal variation, whether in a static cage, an IVC cage, or a continuous stream of air coming from one or more cages. Frequent cage changes and increased cage ventilation dispel the ammonia, but these approaches can be costly in terms of labour and environmental controls. Given these limitations, technology has provided other solutions to help facility managers cope. Generally, they have the common denominator of preventing water from combining with urea to generate ammonia before it's "too late." The various methods include bedding substrates that absorb water, bedding impregnated with water absorbing crystals, autoclaved bedding, increased cage ventilation, humidity control, and wetness detection. The merits of some of these will be discussed in the presentation.

OD9S1 Critical Elements in the Occupational Health and Safety Program: The AAALAC International Perspective

Bayne, Kathryn, Presenting author
AAALAC International

An important aspect of the AAALAC International review of animal care and use programs is the occupational health and safety program (OHSP). AAALAC assesses the OHSP through the narrative information provided in the Program Description prepared by the institution prior to hosting the triennial site visit and during the on-site review. Importantly, the most frequently identified mandatory item for correction identified during AAALAC International accreditation site visits pertains to the OHSP.
The successful OHSP receives strong administrative support as well as input from multiple institutional program units that help to coordinate the OHSP and employs sound implementation strategies. The Guide for the Care and Use of Laboratory Animals (NRC 2011) lists five key elements of an appropriate OHSP: 1) hazard identification and risk assessment; 2) personal hygiene and personal protective equipment (PPE); 3) facilities, procedures, and monitoring; 4) training; and 5) medical evaluation and preventive medicine. Essential components for designing a program that prevent injury and illness in workers due to hazards associated with the animal care and use program include identifying the hazards in the work place—both those associated with the experiment and those intrinsic to working in an animal facility; assessing the risk (or vulnerability) for specific job classifications, as well as of individuals in the workplace; establishing procedures and policies that support avoidance and/or control of exposure to hazards; and providing appropriate health care services to employees. A successful OHSP is developed and implemented with input from both trained human health professionals and veterinarians knowledgeable about animal- and research-specific health risks. The health care services providers should be knowledgeable in the clinical presentation of exposure to identified hazards. In general, a health history evaluation prior to an employee initiating work is a useful tool to evaluate each individual's risk relative to their unique health status. Identifying hazards and the people who are potentially at risk from those hazards, educating individuals about the hazards and the procedures available to prevent exposure, and providing needed health care services relative to the hazards found in the workplace, are the foundation of a successful OHSP. Validation of effective implementation of the OHSP, for example through audits by the oversight body, safety officials, and facility management should be an ongoing process. A summary of the key findings during site visits in each of these categories will be presented to aid institutions in implementing a program that provides effective protection of individuals involved in the animal care and use program and avoids these issues. In addition, a summary of the changes to the revised Program Description (scheduled for release in 2016) regarding occupational health and safety will be described.

OD9S2 Laboratory Animal Allergy (LAA) Program: Risk Based Assessment Process to Control Exposure

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Janssen Research & Development

The concern regarding Laboratory Animal Allergen (LAA) exposure has been demonstrated more extensively the last decade. But how can we monitor the level of exposure in our own facility? Which measures can we take? We have put a program in place to assess the exposure to LAA for our staff and to propose engineering or personnel protection measures.
Our engagement to protect our staff for LAA brought us to develop a program in all our rodent facilities to determine the LAA exposure level for all types of activities where rodents were involved in. Under guidance of Environmental Health & Safety (EH&S), Laboratory Animal Medicine (LAM) and staff of the research groups worked together to gather exposure data to rat and mouse allergens during all kind of activities. Each activity was extensively described according to a Risk Based Exposure Assessment Process (RBEAP) and categorized as high, moderate and low risk activity. The 3 risk categories were associated with levels of 50 ng/m3 (high), based on information described in a J&J Safe Science LLA guideline. Then measurements of Rat Urine Protein (RUP) or Mouse Urine Protein (MUP) in the air were made with small monitoring pumps fixed on the collar of staff (personal measurements) and with monitoring pumps placed close to the assumed exposure location (stationary measurements) during the activity for at least 1 hour. Such measurements were done at least 3 times, during the worst case situations (e.g. in the room just before cage change when the cages are the dirtiest).Air samples were sent to a specialized laboratory for analysis using the Enzyme Linked Immuno Sorbent Assay (ELISA) or the Multiplex Array for Indoor Allergens (MARIA) method. The quantitative measurements were then statistically analysed using a Bayesian Statistical tool (Industrial Hygiene Data Analyst Program).The measured quantitative levels of allergens were then

compared to the assessed qualitative risk levels. This allowed us to get an objective insight in the real exposure to staff and was of great help to decide for an appropriate control program, including a combined engineering/personal protection equipment. Some implemented engineering controls were documented in a LAA Containment Guide sheets for further reference.

OD9S3 Pathogen Contamination of Human Tumors and Patient-derived Xenografts

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Murine models implanted with human tumour cells and patient-derived xenografts (PDX) are being increasingly used for preclinical evaluation of experimental cancer therapeutics. Human tumours and PDX samples may contain human or rodent pathogens. Handling of contaminated human tumours and mice implanted with xenografts may pose a health hazard to laboratory and animal care personnel or research animals.
To assess the potential risk, human tumour and PDX samples submitted to our laboratory were evaluated by real-time PCR assays for the presence of the following human pathogens: 2 strains of Human immunodeficiency virus (HIV1, HIV2), 3 Hepatitis viruses (Hepatitis A, B, C), 2 strains of Human T-lymphotrophic virus (HTLV1, HTLV2), Epstein Barr virus, 3 hantaviruses (Hantaan, Seoul, Sin Nombre), 2 Herpes simplex viruses (HSV1, HSV2), Human cytomegalovirus, 2 Human Herpes viruses (HHV6, HHV8), Human adenoviruses, Varicella zoster virus, and Lymphocytic choriomeningitis virus. A minimum of 1788 samples were evaluated. Results indicated the presence of viral genomic sequences for Epstein virus (2.9%), Hepatitis B (0.6%), Hepatitis C (0.1%), HSV1 (0.1%), Human cytomegalovirus (.01%), HHV6 (0.7%), HHV 8 (0.1%), HIV 1 (1.0%), and HTLV1 (0.1%). A more limited number of samples were evaluated for the presence of 2 Human papillomaviruses (HPV16, HPV18) or the mouse pathogen Corynebacterium bovis. Of the 636 samples tested, 2.2% were positive for HPV16 and 0.6% were positive for HPV18 and of 1,574 samples tested, 4.9% were positive for C. bovis. The results of this study provide data indicating the prevalence of human pathogens in human samples and the need for vigilance by laboratory and animal care personnel in handling human-derived samples or animals implanted with human tumours. I addition, human tissues can serve as a source of contamination for C.bovis, a significant pathogen of the immunodeficient mice used as in vivo models to study these human sample types.

OD9S4 The Human in the animal facility: Keep healthy by awareness and recognition of danger

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Rats and mice execute scientific (behaviour) experiments to study “Stress and Aging”. In this research, the welfare of the animal is essential for the reliability of the results. For my current job I needed to develop new skills like awareness to recognize danger and risks. Now I look with another perspective on the activities of employees in the animal facility. I use these important skills to contribute to improvement of the work circumstances and welfare of the employees.
In the animal facility the focus is aimed at the welfare of the animals. However, more important is the welfare of the employees who are doing their best to achieve this. They are doing the job and are our biggest asset. Beside a good professional climate, an excellent infrastructure of the animal house is essential for good animal caretaking. However, dangers or threats are always present. Whether these can be categorized as high, medium or low risk, depends on the ability to recognize the danger and how to act upon it. Awareness of danger is essential to minimize the risk. Allergy or bite/prick incidents are well known dangers. However, other dangers like bad behaviour or lack of education of the employees are not always recognized. This session will focus on different threats and/or dangers and how you can minimize the risks for the employee. Healthy employees in a good professional climate are the basic for good caretaking of the animals and their welfare. Examples from the past will be used to translate abstract concepts into practical situations and possible solutions. You are invited to contribute with your own experience.

OD10S1 PRIMTRAIN: A COST Action for training and animal behavioural management in non-human primates and other large laboratory animals

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Pauling B, Teepe R, Treue S, Co-authors

Positive Reinforcement Training and Animal Behaviour Management are highly valuable components of state-of-the-art approaches of working with highly cognitive and social animals in biomedical research. The COST Action "PRIMTRAIN" provides a network for personnel working with non-human primates and other large laboratory animals to facilitate the competence and skills needed to successfully apply Positive Reinforcement Training and Animal Behaviour Management.
Positive Reinforcement Training (PRT) and Animal Behavioural Management (ABM) of highly cognitive and social animals used in biomedical experimentation reduce the stress level for the animals, promote more reliable experimental results, facilitate the refinement of methods and procedures and lead to increased safety, both for animals and personnel. However, implementing high quality PRT and ABM, which have substantial and long-term benefits for animal welfare and research, poses a challenge as it requires excellently trained staff. Since research using large animal (including non-human primates) is a small field, opportunities for training on PRT and ABM are rare and it is difficult to network and exchange. Therefore it is important to create opportunities for laboratory staff from different facilities to meet regularly, get educated on ABM and PRT and exchange experience, knowledge and ideas in order to implement the best possible practice in PRT and ABM in their own facility. The aim of the COST Action PRIMTRAIN is to provide such opportunities and create a network in which knowledge and experience are openly shared and state-of-the-art education and training is facilitated. PRIMTRAIN is a network of animal care takers, animal trainers, ABM specialists, ethologists, veterinarians, neuroscientists, and other biomedical researchers using NHP and large laboratory animals from nine different European countries. The Action will offer Workshops, Training Schools, and STSM (short-term scientific missions) for animal laboratory staff. Moreover a catalogue of relevant literature will be compiled and a recommendation of a minimum European standard for all primate and large animal laboratories with regard to animal training will be developed.

OD10S2 Never wrestle with a pig...

Sørensen, Dorte, Presenting author
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The use of positive reinforcement training (PRT) has slowly spread from the world of zoos to laboratory animal facilities housing non-human primates, chimpanzees or dogs. Training our laboratory pigs should be just as common as training the dogs.
According to the EU directive, animal welfare should be given the highest priority. Hence, training of the animals should always be implemented in some form. Possible welfare gain by training the animals will be presented. Pigs (as well as cows, goats etc) can be trained by PRT, but other ways of habituating or luring the animals to cooperate are also possible to implement and often it calls for less work than PRT. Factors that should be considered in all sorts of training are the competences and engagement of the trainers and the equipment used.

OD10S3 Passive desensitisation in non-human primates leads to a better result in operant conditioning

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The use of non-human primates in biomedical research is still needed. However, this should only be done under strict conditions. The BPRC is committed to the best possible housing and husbandry conditions for non-human primates in biomedical research. This includes the use of passive desensitisation and positive reinforcement training.
Positive reinforcement training in laboratory animals is common practice at the Biomedical Primate Research Centre (BPRC). The institute houses selfsustaining breeding colonies of non-human primates (macaques and marmosets) in large naturalistic groups, which gives the possibility to start training animals at a very early age.
Passive desensitisation is a process that precedes operant conditioning. It's the first step for an animals to get acquainted with unknown stimuli that are potentially stressful but necessary in later experimental procedures, thus creating a more predictable environment for the animal. Passive desensitisation shortens the training period and making the selection of well-trained animals easier.
In this presentation, the concept of passive desensitisation will be explained. Examples such as familiarization with personal protective equipment (e.g. mouth masks and gloves) or the presence of a transport box will be given. Including passive desensitization in husbandry routines result in better trainability and thus less stress for the animals in experimental procedures. Over all, this has a positive effect on the well-being of the animals.

OD10S4 Preparing dogs for study life: improving welfare, efficiency and data output

Hall, Laura, Presenting author
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The dog is the preferred non-rodent species in the safety assessment of new compounds. Despite their wide-spread use (>'00,000 used globally each year in research) we know little about their welfare and the impact of routine practices on their welfare. While some form of training is frequently recommended in legislation and guides, there is very little guidance available on effective training protocols. As a result, many practices are based upon anecdotal evidence.
While there is a broad desire to implement effective training for many aspects of laboratory-housed dog use, there remain a number of barriers to uptake, including lack of resources specific to the research environment, lack of confidence in training techniques and concerns about interference with study outputs.
At the outset of a new collaborative project across UK industry, we present evidence-based resources for care, technical and scientific staff to support the implementation of Refinements. Best practice for a number of protocols will be shared.
In this talk, a number of techniques employed to prepare dogs for study life will be presented. These include positive reinforcement training, desensitisation, increased predictability, modified handling and modified dosing techniques. Empirical evidence demonstrating the both the welfare benefits and ease of implementation of an effective, positive training protocol for laboratory-housed dogs are described.
A simple training protocol which can be adapted for several regulated procedures is presented, along with evidence for a lasting effect resulting from a short training intervention. The protocol for a successfully-implemented facility-wide training protocol preparing dogs for study life will be described. As with any planned Refinement, it is necessary to evaluate the effects of a training programme using a validated method. We have developed welfare monitoring tools which can be employed by care staff and technical staff to monitor the impact of planned Refinement on home pen welfare, and to evaluate progress in training.

OE1M1 Provision of Training, Education and Qualification for Animal Care Staff

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Institute of Animal Technology (IAT)

Directive 2010/63/EU Article 23 and 24 (c) which requires that each establishment that breeds, supplies or uses animals has adequately educated, competent and continuously trained animal care staff.
Directive 2010/63/EU states that animal welfare considerations should be given the highest priority in the context of animal keeping, breeding and use for scientific purposes. One of the mechanisms within the Directive to achieve this aim is Article 23, 2. The staff shall be adequately educated and trained before they perform any of the following functions: (a) carrying out procedures on animals; (b) designing procedures and projects; (c) taking care of animals; (d) killing animals.
Article 24 lists specific requirements for personnel, including persons responsible for overseeing the welfare and care of the animals in the establishment.
Educated, trained and competent animal care staff has many benefits for both animals and science, and is essential in maintaining high standards of animal welfare and component of fostering good culture of care within the establishment.

OE1M2 Clinical monitoring in a 45 000 mice breeding facility

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Animal welfare is essential for good-quality animal-based science. However, it is a challenge to ensure health for 45 000 genetically modified mice with potentially multiple phenotypes. For an effective care program, welfare assessment should be part of the daily observation or cage cleaning carried out by the husbandry staff. Staffing should therefore receive suitable training by the attending veterinarian. We present here a visual tool to help caretakers doing this monitoring appropriately.
We have designed a flowchart to manage care staff in animal observation and first care in order to:- Reduce the delay between identification of a sick mouse and the weekly veterinarian visit.- Improve animal care thanks to vet techs (specifically trained technicians who are the veterinarian 1st contact).- Set up quick and effective care and therapy.- Strengthen the compliance to 3Rs (especially Refinement) and to the 2010/063 EU Directive. This diagram consists in decision trees which have been created based on the most frequent pathologies seen in the facility. These trees describe the different symptoms (size, bodyweight, condition etc.) and different actions that animal caretakers have to take when the issue is detected. A colour code and pictures have been added to better visualize the degree of severity. Some technicians who are present every day in the facility, namely Vet Techs, have received a complementary training from the veterinarian to become autonomous to clinically examine an animal and provide appropriate care. Action sheets with more information than trees have been created to help them. In addition to the regular veterinarian visit can be consulted at any moment in case of doubt or clinical problem misunderstanding. Vet techs follow continuing education during the veterinarian's visit and with monthly sessions. The pathology occurrence is also analysed in each room. After a beta-testing in 3 rooms (involving 2 animal caretakers and 1 Vet Tech), this project has been extended to the entire facility (12 housing rooms, 23 caretakers and 5 Vet Techs). Regular meetings are organized to review the training contents and to assess the effectiveness of this clinical monitoring program on the animal health and welfare. Finally, this tool permitted the improvement of:- Skills: animal caretakers have enriched theirs care abilities: more medical vocabulary facilitating communication, clinical examination, new responsibilities, self-confidence... they were also able to manage the usual mouse pathology.- Refinement: Decision trees use has permitted to improve the reactivity when a symptom is detected and to set up adapted care. Communication being more effective (detailed observations, pictures), the veterinarian can give instructions for care and treatments by email if she is not on site. We proposed here a very visual and functional guide. It can be used by all animal caretakers very easily and quickly with a minimum training.

OE2M1 Identifying and overcoming "Grey Zones" arising from the implementation of Directive 2010/63/EU

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EEFPIA, FELASA and ESLAV have joined forces to facilitate implementation of the Directive on new requirements where experience is still limited or that could be interpreted differently ("Grey zones"). A Task Force of experts has already identified several Grey Zones. However, the Task Force would like to involve a wider range of players in this discussion. In this session, Task Force Team will share their work and invite participants to contribute for "Grey Area" identification and addressing.
As a follow up to the 2014 workshop on implementation of Directive 2010/63, EFPIA, FELASA and ESLAV decided to join forces to facilitate and support implementation of the Directive in licensed establishments through development of guidance, in particular, on provisions which set new requirements, where experience is still limited, or in areas where the provisions can be interpreted in different manners (grey zones). A number of volunteers have been identified from within the three constituent organisations and were invited to form small task force sub-groups to identify key questions in their professional network to be included in this survey. Four joint task force sub-groups were set to address: 1. project review/authorisation to include problematic duplication between evaluation and authorisation processes; 2. new functions and competences needed; 3. GA animals (creation, uses, and maintenance including breeding); 4. severity classification (prospective and retrospective), including reuse of animals (formal and welfare aspects). With the following aims: a) define the subject matter (grey zones) including institutions involved (per country); b) identify existing guidance and good practice which are exemplary for resolving any problems identified; c) define information gaps that need to be addressed through additional guidance material; d) develop the guidance in collaboration with other stakeholders as required; e) seek accept-

ance of content by competent authorities through the National Contact Points (NCP) meeting of Member States; f) produce a final information package for dissemination. In this session, the Task Force Team will share the current state of their work and will invite participants to contribute with their own "Grey Areas", as well as proposed approaches and solutions.

OE3M1 European framework for training veterinarians in Laboratory Animal Medicine: Developing a cohesive training approach from Day-One competency to ECLAM Diplomate

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The adoption of Directive 2010/63/EU by the European Parliament and the Council of the European Union has provided leverage for improving animal care and welfare standards and research outcomes at establishments breeding and/or using laboratory animals. The requirement for a Designated Veterinarian (DV) with expertise in Laboratory Animal Medicine (LAM) to be appointed at these establishments whenever possible has implications on the availability of appropriate expertise.
The adoption of Directive 2010/63/EU by the European Parliament and the Council of the European Union has provided leverage for improving animal care and welfare standards and research outcomes at establishments breeding and/or using laboratory animals. The requirement for a Designated Veterinarian (DV) with expertise in Laboratory Animal Medicine (LAM) to be appointed at these establishments whenever possible has implications on the availability of appropriate expertise.

OE3M2 The French veterinary specialization in Laboratory Animal Science and Medicine: a status report

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2FFR. 3Oniris. 4Sanofi. 5Vet-AgroSup. 6Sanofi Pasteur. 7CEA. 8Alfort Veterinary School. 9Domaine de Mirabel, Puylaurens
In 2010, the Directive 2010/63/EU, introduced new requirements for the protection of animals used for scientific purposes. Establishments that breed, supply or use laboratory animals are now required to have a "designated veterinarian with expertise in laboratory animal medicine, or a suitably qualified expert where more appropriate, charged with advisory duties in relation to the well-being and treatment of the animals" (Art. 25 of Directive; Poirier et al.,2015).
In France, the speciality of Laboratory Animal Science and Medicine (LASM) is recognised as such, and listed among the Veterinary Specialities (AM 31/07/2014). First a Specialist level (DESV – "Diplôme d'Etudes Spécialisées Vétérinaires", Specialist veterinary Diploma in LASM) was created in 2001 (AM 28/06/2001), then in 2013 a one-year post graduate program (CEAV – "Certificat d'Etudes Approfondies Vétérinaires" – Advanced Veterinary Certificate in LASM) was set up. The CEAV, designed as a modular program, including post-graduate courses functions a, b, c and d as specified in 2010/63/EU, gives a first level for applying to a specialization curriculum. This certificate can be followed by the DESV. This Diploma allows veterinarians, in France, to use the title of Specialists in LASM (Article R.242-34 of CRPM). Since 2014 these two French levels of post-graduation are offered as continuing professional development (CPD) diploma, for veterinarians employed in various sectors of the profession and aspiring to formal recognition of their competencies in LASM. The assessment of the skills and competencies are based on the learning outcomes of the modular program of the courses, and designed to cover the basic principles of components of a program of veterinary care, specifically in relation to the care and use of animals for research according to 2010/63/EU requirements (http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf). So far (period 2014-2015), a total of 36 veterinarians have submitted their credentials for this specialty; 20 at CEAV level and 16 at DESV level. Of these, respectively 15 and 14 passed the examination on the first examination, whereas other candidates had to complete the curriculum by following one or several training modules in order to satisfy the requisites. A recognition by National Competent Authorities (NCA) of Laboratory Animals Veterinarians, with a specific specialty training in the field is essential, as these individuals are key persons to ensure health and welfare of animals and adequate and responsible use of them in research and therefore contributing to the advancement of medical and scientific knowledge under conditions acceptable to the general public, and in agreement with 2010/63/EU. We are confident that our national post-graduate program will also allow an ECLAM approved residency program in France as soon as possible, and thus an extra asset for applying for the ECLAM Diploma.

OE3M3 The FELASA accredited online educational programme for the competent designated veterinarian in laboratory animal facilities

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Abelson, Klas[1], Co-Author, Bratbo Sørensen, Dorte[1], Co-Author, Østergaard, Grete[1], Co-Author

The LabVet Europe programme aims at qualifying designated laboratory animal veterinarians to the level demanded by the [2]0[1]0 EU directive on the protection of animals used for scientific purposes and the subsequent guidance paper. It is to be seen as continuation of the competencies gained from following the previous FELASA category D programmes, but it is solely dedicated to those thousands of vets who work world-wide as designated laboratory animal veterinarians.
The laboratory animal science community is at present faced with new challenges to meet the objectives of the new Directive 2010/63/EU. This can be seen as an opportunity to further advance laboratory animal science training and education. One of the challenges is that the Directive has clear demands for education of designated vets as defined by the learning outcomes of the guidance paper. Adhering to these will bring the authorized veterinarian to a level acceptable for fulfilling the tasks in a laboratory animal facility. The LabVet Europe programme from the University of Copenhagen has been accredited by FELASA for fulfilling these learning outcomes. As the specific learning outcomes for designated vets in the EU guidance paper are mainly theoretical the programme is 100 % online with no need for travel. To enter the program in addition to a DVM degree, one need to have two years of working experience and to fulfil the learning outcomes for EU function A, B and D. The programme contains supervised teaching for 30 ECTS and is finalized by a written and an oral exam, as well as some assessments during the program. For those interested, the program director will assist the candidates into an alternate route to the ECLAM exam. Having gained access to the program the candidates will have a life-long access to the European LabVet community; an online and confidential platform where information can be exchanged between laboratory animal vets. For applicants not possessing function A, B and D skills an online version of the University Copenhagen FELASA accredited function A/B/D course is offered, which, however, includes a two-day visit to Copenhagen. For those simply interested in watching and using some of the teaching materials, there is a low-fee access available, which, however, does not include supervision and certification. For more information, visit http://labveteurope.ku.dk/

OE5S1 Best practices and implementation of the 3Rs in FELASA accredited courses based on an audits' retrospective analysis

Kolf-Clauw, Martine, Presenting author
FELASA Accreditation Board for Education and Training
Berdoy M, Dontas I, Nevalainen T, Santos AI, Sjöquist M, Gyger M, Co-authors

Course audits are key-elements of the FELASA accreditation scheme, going towards good practice in LAS education and training. The best practices in accredited courses will be presented, based on the analysis and recommendations from twenty audits reports of FELASA accredited courses. A special focus will be made on the 3Rs in the course programme, demanding ethical approval of the procedures done on living animals. A concrete and convincing argumentation showing how 3Rs are applied should be given.
In practice, the teaching process should be based only on best practice. Replacement of live animals by models or dead animals should be implemented for some procedures. Reduction principles should be applied and clearly indicated to the participants. The use of surplus animals and animals shared for other pedagogic or scientific purposes instead of using another set of animals has been recommended. Refining implies a close and direct supervision by competent trainers and the use of analgesia and anaesthesia for animals in the training of procedures. A detailed checklist for all procedures is needed. The staff student ratio during these classes is a criteria focusing major attention. An individual register of all practical procedures should be clear, consistent and understandable so that it can be independently verified. A special attention is given to certificates: the species mentioned in the practical section of the certificate must reflect what was covered during the sessions.
For general organization, implementing formal teaching meetings with formal minutes, used in conjunction with a summary of the principal findings of the evaluations by students will continuously improve the effectiveness of course delivery.
The course organizers should provide a formal circulation of minutes after the trainers meeting. The exams should cover all topics and assess general knowledge and principles.

OE5S2 FELASA Accreditation of Education and Training Courses in Laboratory Animal Science: what does the past tell us and how will the future look like

Gyger M, Kolf-Clauw M, Santos AI, Mohr B, Tolba R, Presenting authors
[1]FELASA Accreditation Board for Education & Training
Berdoy M[1], Dontas I[1], Sjöquist M[1], Co-Authors

The first recommendations for accreditation of courses in animal experimentation were published in 2002. The accreditation board for education & training came into force in 2003 and it introduced audits in 2006. Since 2010, the legal landscape of education and training has, of course, changed drastically.
During this session we will visit the past by presenting the first retrospective study of audits under the category accreditation scheme and try to understand what the past can tell us for the future of accreditation. We will present an update of the FELASA accreditation scheme under the EU legal framework, future developments and how the competent authorities assess the FELASA approach to education and training. We will give an example of an academic institution's quality management of education & training and finally how the FELASA approach can be useful to other legal landscapes outside of Europe.

OE5S3 The impact and perception of FELASA Accreditation in Europe

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Berdoy M, Dontas I, Kolf-Clauw M, Nevalainen T, Sjöquist M, Gyger M, Co-authors

The Federation of Laboratory Animal Science Associations (FELASA) accreditation system for Education and Training (E&T) has become recognized as a robust way of improving training and at the same time establishes the new European golden standard for LAS education and training.
FELASA accreditation aims to implement best practices for both high quality science and improved animal welfare by recognizing, supporting and enhancing the quality of training. It establishes a more uniform standard of competence of the trainees and enables the identification and sharing of good practice. The process has evolved and adapted over the years to provide an independent reassurance for National Authorities and the public about the competence of those working with laboratory animals. An added value of accreditation is that it facilitates the mobility of scientists that are well educated and trained in Laboratory Animal Science within Europe and beyond.
Although the FELASA accreditation scheme incurs some financial and time commitment for course organisers numerous applications have been received and evaluated since 2003. The balance of the impact of the FELASA Accreditation scheme in the scientific community will be presented not only in quality but also in numbers to illustrate the success of the scheme.
National Competent Authorities constitute another interest group of the accreditation system. Their perception of FELASA accreditation is therefore crucial as a part of the enforcement of the EU Directive 2010/63 at national level. FELASA has launched a survey through its constituent Laboratory Animal Science Associations to help assess how competent authorities perceive the FELASA accreditation scheme. The results suggest that more than half of the replies confirmed that individuals presenting certificates from FELASA accredited courses have been recognized as competent and are only required to follow national legal modules. The competent authorities of a few European countries also accept FELASA accredited courses from other countries as equivalent to the ones provided by their own country. This is a convincing endorsement of FELASA's commitment to the E&T Accreditation scheme.

OE5S5 One World – The Influence of Shared European Values on Laboratory Animal Science in Africa: Harmonisation of Ethics, Standards, Education, Training, Accreditation and Regulated Procedures – The Example of South Africa

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Africa is a large geographical region containing diverse countries, cultures and languages. Several initiatives aim to harmonise ethical, animal welfare and competency standards in laboratory animal science across the continent. Though Africa will always have its unique strengths and applications, there is much common ground with shared European values. With South Africa as example, it is shown how relevant European philosophies can be adopted or adapted to the benefit of science and welfare.
International guiding principles for the care and use of animals for scientific purposes include those released by the World Organization for Animal Health (OIE), the Council for International Organizations for Medical Science (CIOMS) and the International Council for Laboratory Animal Science (ICLAS). Though African countries and organisations may adopt these standards and best practice recommendations, the full implementation thereof remains challenging due to the absence of specific legislation governing the care and use of animals for scientific purposes, resource and infrastructure constraints of developing economies, the absence of a critical mass of experts in many regions and cultural views of animals in society. The need for practical, implementable solutions is clear, particularly in education and training. In order to establish the education and training needs in laboratory animal science in Southern Africa and to develop solutions to address these needs, the South African Association for Laboratory Animal Science (SAALAS) convened a conference in 2015, attended by 83 delegates (of which 11 internationals). Consideration was given to the education and training of all people in the field. Solutions were approached in the context of national and African needs, accreditation and quality standards, resources, animal welfare and the 3Rs.International experts informed on best practice standards, including ICLAS, the Federation of European Laboratory Animal Science Associations (FELASA), the Institute of Animal Technology (IAT), the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) and Royal Society for the Prevention of Cruelty to Animals (RSPCA). There was strong agreement that South African education and training should be based on international standards, including the establishment of FELASA-accredited courses in South Africa, IAT courses for technologists, "train the trainer" workshops, accredited continuing professional development, courses for animal ethics committees, training in animal welfare, the 3Rs, and wildlife courses. SAALAS working groups with international partners will help drive these collaborative programmes. Overall, European standards were agreed to set appropriate and relevant standards for the South African context. A pan-African initiative is needed to ensure high quality education, training and competency in laboratory animal science on the continent. Partners are invited to contribute.

OE6S1 A Common European Education and Training Framework – is Mutual Recognition Achievable?

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A laboratory animal science (LAS) education and training (E&T) framework has been developed by the EU to respond to the need for harmonization and a common approach to ensure competence and to facilitate free movement of personnel. The details are contained in an EU working document that was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU at their meeting of February 2014.
It is published at: http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htmWithin this document, one topic covered is that of approval and accreditation of education and training courses. However, the EU working document does not differentiate between processes of approval and accreditation and sets out requirements that could be applicable to both. Whilst this might be a desirable solution in the long-term, there is the need to address the current situation and develop a set of principles and information requirements that would allow mutual recognition of E&T courses between Member States. There is agreement by Member States that there needs to be a common approach to assure confidence in the quality of E&T and the assessments being provided. Lack of mutual recognition of E&T courses within the EU could lead to animals used unnecessarily for training purposes if training has to be repeated. Free movement of personnel could also be hindered. Since arrangements for LAS education and training is at the discretion of the individual Member State, there is the risk that different requirements for assuring the quality of E & T courses may arise, particularly since those proposed in the EU working document could be seen as aspirational. This

could lead to a variable quality of animal welfare and the science using live animals. ETPLAS has developed minimum information requirements for mutual recognition of E&T courses. These requirements have been agreed by the National Contact Points and the document describing the ETPLAS initiative will be linked to the EU endorsed working document.

OE6S2 NCLASET – Nordic Consortium for LAS Education and Training

Matti, Nikkola, Presenting author
Nordic Consortium of Laboratory Animal Science Education and Training (NCLASET)

The consortium NCLASET was founded as an LAS Education and Training co-operative to help the member organisations meet the requirements initially formulated in the European Directive 2010/63/EU. The present members include Karolinska Institutet (also the base of the consortium), University of Lund, University of Gothenburg, Umeå University, Uppsala University, Stockholm University, Swedish University of Agricultural Sciences, University of Karlstad, University of Bergen and AstraZeneca.
The aim of this co-operative is to provide education and training that - support Mutual Recognition and Accreditation of LAS education and training- is organised on an on-demand basis, with continuous entry to courses- supports education and training for all functions as specified in the EU directive- supports education and training for all species used in research that requires training - is individualised according to the needs of the course participants- is harmonised to ensure an even high quality level- is based on existing educational resources- is cost-effective to focus available resources on continuous development of course materials and courses- provides a modular reference for clear Continuous Professional Development (CPD) programs, from vocational programs in secondary education to academia. The non-profit co-operative was launched in the Autumn of 2012 and the formal contract outlining the consortium was signed in 2014. The consortium has an educational web platform, where the jointly developed and maintained theoretical course modules are served. These course modules are used in the Education and Training activities organized by the individual parties. Both theoretical and practical course modules are defined with contents, Learning Outcomes and Examination Criteria, following the pedagogic principle of Constructive Alignment. The standard language is English. Learning Outcomes are secured through on-line self-assessment tests connected to each course module, with mutually accepted examination criteria. The following course modules are in use: "Swedish Law and Ethics", "Rodents and Lagomorphs", "Fish", "Non-human primates" and "Aquatic amphibians". New course modules in development include "Wild birds", "Dog", "Pig", "Experimental fishing", "Terrestrial amphibians and reptiles". Additional course modules are planned. The number of people taking course modules in 2015 was 1300. The modules have been designed to separate general biological content from any specific national legislation, so that the course modules can be utilized in different countries. More information of the co-operative will be found at the consortium website NCLASET.org, which will provide information about the course modules developed within the consortium, and the different courses provided by the members in the consortium. We actively seek and welcome collaboration and co-operation in our activities in education and training, such as partnering with the consortium.

OE6S3 Teaching, training and assessment of practical skills - the challenge of harmonisation

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University of Copenhagen

Mutual recognition of competences obtained at various courses around Europe is challenging, especially with regards to practical skills. Thus, there is need for reliable tools for teaching, training and assessing practical skills, which can facilitate comparison of the practical skills of an individual.
While theoretical knowledge is fairly easy to assess, it is far more difficult to judge a person's practical skills obtained at an educational institute. This may have consequences for persons aiming at performing animal experiments in European countries other than that where the basic training was conducted, since the basic skills as well as the need for CPD activities are difficult to evaluate by the competent authority. At our university, we have implemented a system for assessing the learning outcomes and technical skills of the student's in practical exercises with live animals that is simple to implement and has been well perceived by the students.

OE6S4 Education in Primate Research: The EUPRIM-Net Laboratory Animal Science Course

Stephan, Valeska Marija, Presenting author
German Primate Center

Working with non-human primates in the laboratory demands a particular education and training. Our course "Laboratory Animal Science Course on Primates" follows the most recent guidelines from the EU Directive and the FELASA and aims to provide a solid educational foundation for personnel working in primate research.
Advancement of scientific knowledge through biological and biomedical research is critical for our understanding of human and animal physiology and consequently for medical progress. Where no alternatives exist, this research necessitates the use of non-human primates (NHPs) currently and for the foreseeable future. The EU-funded research infrastructure project EUPRIM-Net brings together the unique facilities and solid experience of ten European publicly-funded primate centers, in order to meet the highest ethical standards in using these sentient animals while ensuring top-level research. To ensure that NHPs receive adequate care and are handled correctly, all personnel involved in the work with primates have to receive appropriate education and training. In order to lay out a solid foundation for education of personnel in primate research we developed a course on Laboratory Animal Science which aims in particular on an audience, which is less experienced in the work with NHPs. It adapts the most recent education guidelines from the EU Directive and the FELASA (for functions A&B) and their specific learning outcomes and provides a standardized fundamental education and training. To ensure the best and most effective learning progress topics and content have been separated into an e-learning component and an on-site component. While the e-learning component covers the important theoretical frameworks (e.g. legal aspects), the on-site component focuses on more practical based content (e.g. methods in primate research). The on-site component is a week long course and is held at German Primate Center in Goettingen/Germany. Working with NHPs in research raises strong ethical points. Hence our course puts a particular focus on ethical and animal welfare aspects in order to sensitize the participants for these issues, their responsibilities towards the animals in their care and encourage critical thinking and evaluation of their own work at any stage along their careers as a scientists working with NHPs. To date the course has been held three times with participants from Germany and other European countries.

OE7W1 E-learning resources to meet the requirements of the EU 2010/63

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EU Directive 2010/63 requires those undertaking research procedures that involve the use of laboratory animals to undergo appropriate training. Assessment of this training and of their competencies, together with advances in the 3Rs, will require regular updating of this initial training. Delivery of training to this large number of individuals (>16,000 in the UK) is already resource intensive, and the required time and effort of trainers is likely to increase. Provision of e-learning modules to support this training can help with meeting the requirements of the Directive in a cost-effective way. As an initial contribution, with support from the UK NC3Rs, we have delivered two modules (EU 20 and EU 5) and others (EU 6 & EU 21) are in preparation. The modules are produced in Articulate Storyline, and delivered both as standalone modules (www.nc3rs.org.uk/our-resources) and via an LMS (Learning Management System) which enables tracking of the progress of individuals, and updating of their Institute's training officer with information on module completion (www.flairelearning.com). At present, this resource is delivered free of charge, although registration is required for tracking. The modules make use of scenario-based learning, and incorporate video, still images and interactive animations to help engage and inform the user. The modules have been structured to meet the learning outcomes required for training developed under Directive 2010/63/EU. Version 1 of the modules deal predominantly with rodents, but content relevant to other species is also included, and this will be expanded in future releases.

OE7W2 Meet the need: education for laboratory animal science as recommended by the EU using the online learning tool LAS interactive

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The well-being of animals used in animal experiments and the validity of scientific results depend to a great extent on the competence of all personnel involved as defined in Art. 23 of Directive 2010/63/EU. Therefore, any person involved in animal research has to be adequately educated, competent and continuously trained. At the same time the 3R concept claims a reduction and refinement of animals used in education.
According to the EU recommendations (1) targeted learning modules ensure basic as well as species or function and task specific training with a focus on the 3Rs. LAS interactive is an online learning platform that meets this challenge. Our learning content is developed in close cooperation with experts from several universities and institutions such as the German primate center or the May Planck Society for Ornithology. The implementation of videos, slide shows, interactive tools and exercise questions contribute to the 3Rs since they prepare trainees as best as possible before starting practical training. LAS interactive is divided in two sections. "vtk online" is our free information portal for laboratory animal science funded by the German Research Foundation. Our "las campus" provides online courses and continued professional development modules both to attain theoretical qualification according to EU recommendation and national law. Courses are available on demand for members of institutions which are involved in working with research animals or performing animal experiments. A course typically consists of a general part (EU core module 1 - 6) and the relevant species-specific part. The content can be adapted to country specific requirements and can be supplemented with information relevant for the institution. The online availability of LAS interactive, consisting of a multilingual free information portal structured according to EU recommendations, alongside with multilingual online up to date education modules will contribute to harmonize education in laboratory animal science throughout the EU and therefore to promote the 3R.

OE8M1 The Use of Performance Standards for the Accreditation of Animal Care and Use Programmes

Guillen, Javier, Presenting author
AAALAC International

The AAALAC International accreditation of animal care and use programmes involves the evaluation of all programme areas in diverse research, cultural, financial and legal environments. In such variety of frameworks that may require different engineering standards, the performance-based approach is essential to maintain consistency of the accreditation standards. This evaluation approach focuses on well-defined desired outcomes regardless of the processes used to achieve them.
AAALAC International has accredited almost 1,000 animal care and use programmes in 41 countries. Implementation of consistent accreditation standards is a challenge, since programmes may differ in type of research, species, institutional organisation, cultural traditions, financial means, and applicable legal frameworks. Some implementing laws and guidelines establish requirements or recommendations containing engineering standards, which are objective and measurable, but not flexible (e.g.: minimum cage sizes and animal densities). However, accreditation is not only based on compliance with applicable legislation, but also on other standards that in some cases may go beyond those required by law and that can be applied in diverse research environments. In addition to applicable legislation, programmes are expected to comply with performance standards of the three AAALAC Primary Standards: The Guide, the ETS 123 and the Ag. Guide. Performance standards are outcome oriented, focused on goals or expected results rather than the process used to achieve the results. They allow flexibility to fit different situations, but sound professional judgement is needed to evaluate their application. They need the outcome to be defined in detail, and a process to evaluate the efficacy of the process implemented. The performance approach can be applied to many programme areas. As an example concerning a fundamental area such as the ethical review process, AAALAC accepts different ways of performing programme oversight and ethical review of animal experiments. Composition and functions assigned to oversight and/or ethical review bodies differ according to different legislations (different engineering standards), so AAALAC focuses on the outcome of the process: the quality of the oversight and ethical review applied in practice, which can be evaluated by the observations during the evaluation visits. A more simple example can be the sanitisation of cages: AAALAC does not care if cages are washed in sophisticated automatic equipment or manually. What AAALAC will evaluate is if there is a procedure in place to regularly monitor the efficacy of the washing process, regardless of the process used. Examples of performance standards will be discussed.

OE8M2 Global Harmonization of Performance-Based Standards in a Contract Research Organization

Anderson, Lynn, Presenting author
Covance

Covance runs animal care and use programs in North America, Europe and China. Despite differences in regulations and cultural norms, Covance has a common Code of Respect for Animals in Research and Development to accept the legal and moral obligations to assure that animals are treated in accordance with all applicable rules and with high standards of respect and compassion. All of our sites are AAALAC accredited and use the Guide for the Care and Use of Laboratory Animals as our global standard.
Harmonization of various aspects of the animal care and use program helps to provide consistency across sites and minimize variables during the execution of studies that may be conducted at more than one location. Activities that lend themselves to global harmonization can be broadly categorized into four areas: personnel management, animal care and veterinary medical management, IACUC or equivalent animal welfare bodies, and facility operations. Specific targets for harmonization include animal welfare training, use of personal protective equipment, biosecurity, rodent sentinel monitoring, social housing, environmental enrichment, acclimation to procedures and restraint, blood and dosing volumes and techniques, water quality testing and HVAC performance. The harmonization process begins with agreement on the interpretation of global standards, such as the Guide for the Care and Use of Laboratory Animals. Institutional policies and guidelines can then be developed to document the institution's expectations for various aspects of their animal care and use programs, regardless of location. These policies and guidelines must also allow for the differences in country-specific regulations and flexibility in the application of site-specific procedures. While the specific processes or methods to achieve the standard may vary slightly from one site to the next, the guidance document will establish performance expectations and provide the general direction for the activity. Covance has harmonized a number of processes by using working groups (WG) with cross-functional representation from all impacted sites. Each WG begins by establishing a charter that identifies their objective, the scope of their project, the deliverables, the activities or tasks to be performed, the benefits of the project, critical success factors and key stakeholders. The first WG activity is to collect baseline data from each site. Next, the regulatory basis and standards for each site's practices should be identified and then the project team should conduct a gap analysis. This gap analysis should drive discussion leading to best practices and establishment of the guidelines and minimal standards of care for each site. Once the stakeholders have approved the guidelines, personnel are provided training. The goal of harmonization should be to minimize variables to support quality science, reliable data and animal health and welfare.

OE8M3 Implementation of performance standards as key drivers for improving animal welfare and harmonizing practices in the pharmaceutical industry

Solís, Violeta, Presenting author
GSK

Animal work is conducted globally at GSK, with varying laws and differing cultural views. Thus, we have a worldwide Policy on Care, Welfare and Treatment of Animals, which establishes principles and expectations, and includes the purpose to maintain active AAALAC accreditation for all animal facilities and animal care programmes throughout the world
GSK endorses the principles of professional judgment, performance standards and harmonized approaches to animal work, and is committed to promoting the care, welfare and treatment of animals in research and testing. Therefore, the company has a department driving the strategy for animal welfare and ethics, and also dedicated quality groups. Oversight of animal care and use programmes is accomplished through enterprise level governance and a risk management framework. This framework includes the requirement for management monitoring, independent business monitoring and corporate audits. External oversight is accomplished through visits by regulators and external assessment, e.g. AAALAC. One valuable resource we have at the management monitoring level is the cross-site visit. In these visits, staff from different animal facilities visit and evaluate each other's facilities and practices. These are informal assessments that aim to support mutual improvement thought a "fresh pair of eyes" approach and can be used to aid in preparation for AAALAC site visits as well as regulatory inspections. Additionally, we have established a working group named "Community of Practice for Animal Welfare" with the objectives of sharing practices and promoting and supporting animal welfare initiatives. One of the main activities of this group has been to establish behavioural performance standards for species we house internally and with collaborators. These behavioural standards define the key behaviours any given species should be able to exhibit and therefore should be encouraged at each of the sites. This means we have harmonized housing requirements, but each region has the flexibility to achieve the expected outcome based on local resources and layouts. Developing performance standards has helped us build a strong team of people who act as a conduit to gain resources for our enrichment programs, gain support for evaluating our housing and environmental enrichment, and help prioritize enrichment strategies. So far behavioural performance standards have been established for mice, rats, guinea pigs, rabbits, mini pigs, dogs and macaques. Examples of improvements achieved from this initiative will be shown for different species.

OE8M4 Accreditation of animal care and use to leverage the harmonization of animal welfare

Decelle, Thierry, Presenting author
SANOFI

Sanofi conducts animal research in 30 sites in 10 countries. Sites are originated from different companies, divisions (pharmaceuticals, vaccines, animal health), and functions (Industrial Affairs, R&D). Countries have their own regulations, and culture. To ensure Good Animal Welfare Practices, a corporate policy on the Protection of Animals under the Corporate Social Responsibility, the Bioethics Committee and the Chief Veterinary Office has been developed to endorse consistent ethical tenets.
Regarding ethical values, in a global company, there are no reasons to consider animals used in research and testing in the different regions at different levels of importance: consistent standards should be applied across the sites. Although, there is no willingness to work on standardization, which would be more considered as the implementation of rigid engineered standards, we should be able to demonstrate the absence of ethics dumping (Reducing the risk of exporting non ethical practices to third countries). To manage all aspects of the scientific projects, including the local constraints, the local animal care and use programmes must rely on flexibility to fit different situations. Sound professional judgment is needed to evaluate the performance of all aspects of the programmes and the compliance with the Policy. AAALAC International accreditation of animal care and use programme is one of the means to ensure compliance with minimal standards. Accreditation is not an objective per se as it is considered as a peer-review step to support continuous efforts and to improve every programme: accreditation relies not only on regulations, but also on standards that may go beyond those required by law and that can be applied in diverse research and testing environments. By seeking and maintaining accreditation, harmonization of animal welfare is enhanced. Organizations and systems, including AAALAC accreditation, have been set up within Sanofi to ensure consistency of all animal welfare programmes. Roadmap for the Protection of Animals will be discussed during the presentation.

OE9S1 NC3Rs: Pioneering better science through application of the 3Rs

Lidster, Katie, Presenting author
NC3Rs

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is a UK organisation which leads the discovery and application of new technologies and approaches to replace, reduce and refine the use of animals for scientific purposes. To deliver this mission we have adopted a diverse strategy which includes funding 3Rs research, providing a wide range of 3Rs resources and supporting open innovation in 3Rs technology development.
The NC3Rs funds 3Rs research, training and career development, supports open innovation and commercialisation of 3Rs technologies, and stimulates changes in policy, regulations and practice relating to the use of animals. We collaborate with research funders, academia, industry and regulatory authorities to promote robust and ethical scientific practice. As part of our remit, we provide an extensive library of 3Rs resources including guidelines, practical information, publications, videos and training materials. Our resources are used nationally and internationally to help researchers, veterinarian and animal care staff put the 3Rs into practice. For example, the ARRIVE guidelines are endorsed by over 700 journals and over 10,000 copies have been disseminated to researchers in 39 countries. Following on from the ARRIVE guidelines, we have recently launched a new online tool - the Experimental Design Assistant (EDA) - to guide researchers through the design of their experiments and improve the internal validity of animal studies.
This presentation will provide an overview of the NC3Rs; who we are, what we do and how we use a science-led and evidence-based approach for driving 3Rs advances. This will include an overview of our research funding schemes and an introduction to our expanding library of 3Rs resources and how these can be used to implement the 3Rs. Further information about NC3Rs activities and programmes can be found at www.nc3rs.org.uk.

OE9S2 A presentation of the Danish 3R-Center, its organisation, mission, tasks, projects and future plans, with a special focus on the funding of 3R-research project

Bengtsen, Tom, Presenting author
Danish 3R Center

The Danish Ministry of Food, the pharmaceutical industry and a number of animal welfare organisations in Denmark agreed in the spring of 2013 to establish the Danish 3R-Center with a scientific board, a budget of its own, research funding and a secretariat.
It is the goal of the Danish 3R-Centre to generate a leading environment within the implementation and dissemination of the 3Rs, with a mission to initiate activities that may lead to the immediate implementation of the 3Rs, provide a forum for collaboration, discussion, exchange and dissemination of information on the 3Rs and to initiate research projects and recommend funds allocation of resources within the area. The 3R - Center has developed a web site in both English and Danish aiming to be a 3R-news hub for relevant international 3R news. At the web site you can also find educational material focusing upon high school students at A-level biology, bio-chemistry and bio-technology. Other projects as a survey on the 3R-knowledge in the scientific world and the annual 3R rapport can also be found. Furthermore the Danish 3R-Center hosts a two day scientific symposium every year with both Danish and international presenters and participants. Finally the Danish 3R-center funds 3R-research projects with at total sum of 200.000 € per year. Currently 10 projects have been funded. These will be presented along with future plans.

OE9S3 Norecopa: Guidelines, databases and other 3R-resources for animal research

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With the implementation of EU Directive 2010/63 and global interest in laboratory animal welfare and alternatives, the need for more species-specific guidelines has never been greater.
Finding these guidelines in the ocean of scientific literature is not always easy. Searching for them is often hindered by the fact that many scientists do not highlight progress within the 3Rs in their publications, or do not publish all the small details of a procedure which are often critical for its success. One part of the solution to this challenge is to ensure that laboratory animal specialists have easy access to guidelines, information centres, discussion forums, journals, key publications and databases containing information of relevance to the 3Rs.Norecopa and the US Department of Agriculture's Animal Welfare Information Center (AWIC) have compiled a global database containing such information, called 3R Guide. In addition, Norecopa has also developed an intelligent search engine using a variety of techniques to help users find relevant material, not only in the 3R Guide database but also in Norecopa's other databases and on its webpages. To make these resources readily available for all user groups, Norecopa has constructed a totally new website, bringing together all its databases, global 3R-resources and the new search engine: www.norecopa.noNote to referee panel: this new website has not been launched yet but will be available in good time by the FELASA Congress

OE9S4 EPAA, a partnership to facilitate regulatory acceptance of alternative methods

Dal Negro, Gianni, Presenting author
GlaxoSmithKline

In the past ten years, Europe saw emergence of a growing number of initiatives pledging for the development of novel alternative methods to animal testing for regulatory purposes. Scientific progress, modern societal and ethical values, as well as legal framework on the protection of laboratory animals (Directive 2010/63EU) and other sectorial regulations, have paved the way for this concerted effort to foster the development and acceptance of alternative methods

Despite the significant continued efforts and investments by the public and the private sectors, there are still some scientific challenges that do not allow fully replacing animals in all toxicity areas. Another reason is that it is a long journey from fundamental research to regulatory acceptance. The European Partnership for Alternative Approaches to Animal Testing (EPAA) helps in addressing these challenges. The presentation will showcase how this partnership between regulators, and 7 regulated industry sectors in co-operation with many other interested parties supports the identification of scientific gaps, and fosters development, promotion and regulatory acceptance of 3Rs approaches.

OE4S1 Memorandum of Understanding between Air France and the French public and private research institutions: a joint agreement for the continuous improvement of transport of 'biological resources' for research purposes

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Bruno Verschuere1, co-author, Nicolas Dudoignon[1], co-author

Air France continued decision to transport laboratory animals, particularly primates, is due to long lasting and efficient relationships established between the company and representatives from French research, at different levels. For instance, written support from renowned researchers was key to reassure the airline of its social responsibility related to transporting animals for research purposes.
However, with the growing intensity of the intimidation campaign strengthened alliances were needed, including additional support from the national authorities.
In the years 2013-2014, after several years of resistance to the pressure imposed by animal right groups and with the continued willingness to transport primates for their research purposes, Air France was asking for additional support from beyond the research community, especially from the national authorities. At the same time, GIRCOR liaised with the French Research Ministry suggesting them to take a strong action in support of the air transportation of laboratory animals.
Subsequently, the decision was taken to create a permanent working group aiming at improving and securing the air transport of biological resources that are necessary for medical, veterinary or biomedical research. It was agreed that this should be managed through a Memorandum of Understanding (MoU) that would not be limited to the common live laboratory animals, but would widely and indifferently cover "any biological specimen" (animal, plant, microorganism), dead or living.
After a 1st meeting organised in the offices of the Research Ministry in December 2014, with representatives (including lawyers) of Air France, of the Research Ministry and of public and private research institutions, a first draft of the MoU was approved and prepared for signature.
The finalised MoU was signed in September 2015 by the Research Ministry, Air France, the eight major public research French institutions and the three major private research French professional unions. This was shortly followed by the first meeting of a steering committee in order to further discuss the content, the priorities and the way forward.
The presentation will focus more in details on the content of the MoU and the items prioritised by the steering committee members.

OE4S2 Primate Transport: Refining the animals' experience

P.E. Honess, Presenting author
Bioculture Group, Mauritius

The development of significant primate research facilities in non-habitat countries and the limited capability to locally supply animals from ex situ breeding facilities has meant that biomedical research has relied on supply from the wild and in situ breeding facilities to meet its research and testing needs. This has led to concerns about sustainability of wild primate populations as well as the costs of ex situ breeding. Inevitably this has been accompanied by a debate about the welfare impact of transporting animals over significant distances.
This debate about the welfare impact of transporting animals is now more informed as a result of focused research and a need to consider the lifetime experience of the animal and for securing the quality of the scientific models these primates represent. In particular considerable efforts have been made to refine the preparation of animals for transport and the transport process itself to address some of the welfare issues of transport and these are reviewed in this presentation. While organisations and funders involved in animal research have a primary concern about the continuity of supply, the welfare impact of the transport process must be balanced against high quality living conditions under ambient tropical climates that it is possible to provide at high quality in situ breeding facilities. This presentation draws together many of the variables mentioned above with a view to enabling a balanced evaluation of animal welfare issues and continuity of supply related to the provision of primates for well-justified programmes of research.

OE4S3 The Last Airline Flying

K. Leech, Presenting author
European Animal Research Association

Campaigns against airlines, organized by groups like PETA and CFI, are the epicenter of a growing celebrity led crusade to halt the transportation of research animals. Air France is the only commercial airline still prepared, even with the ongoing campaigns against it, to transport research primates for biomedical research. What role does the scientific community have in mobilising public and political opinion to support those transport companies still flying research animals, and help to convince new companies to join this life saving endeavor?
Campaigns against airlines such as Air France, organized by groups like People for the Ethical Treatment of Animals (PETA) and Cruelty Free International (CFI), are the epicentre of a growing celebrity led crusade to halt the transportation of research animals; in particular NHPs and dogs. Air France is the only commercial airline still prepared to transport research primates for biomedical research. Such high profile and celebrity led campaigns have proven to be very successful in forcing airlines out of the business of transporting animals. PETA recently claimed that, "With no way to cheaply and easily obtain monkeys from abroad many laboratories will have a difficult, if not impossible, time getting their hands on animals to torment in cruel and archaic tests".
However, let's imagine for a moment that this celebrity led crusade began in the 1980s. That activists had been successful in halting the global transport of NHPs and dogs, what advances in scientific understanding, and what discoveries involving dogs and NHPs could we potentially not have today? Here are a few examples:
1) NHP and mouse models have been key to developing anti-retroviral drugs that help prevent transmission of HIV and improve and extend the lives of those infected with the virus.
2) Because dogs' heart physiology and anatomy closely resembles humans, dogs are especially suitable for research on cardiovascular diseases. Electrical defibrillators, some heart medications and blood transfusion procedures exist today thanks to research using dogs.

3) The development of RTS,S (MosquirixTM), a candidate vaccine for malaria, was built on 30 years of research using mice and monkeys.
4) Many breakthroughs in treatment for Parkinson's disease have relied on animal research. Current drug therapies and tremor-reducing deep brain stimulation have transformed patients' lives and could not have been developed without research using non-human primates.
If all airlines had capitulated in the 1980s in the way that PETA and CFI have successfully achieved with all but Air France in the past decade, then our understanding of diseases like Parkinson's, Alzheimer's, HIV, and malaria would be years behind where they are now.
Studies in dogs and NHP's form a small but vital part of the research and development process required to develop new medical treatments for human diseases. More people are alive, living longer and with improved quality of life thanks to medical advances which would not have been possible without animal research. It is to the credit of Air France that they continue to transport NHPs and other research animals. The research community in the US and Europe should make vocal their thanks to Air France and encourage other transport providers to take part in this life saving endeavour.

OF1M1 Animal Research: Time to Talk!

Leech, Kirk, Presenting author
European Animal Research Association

The European Animal Research Association has been established to inform the European public on the continued need for, and benefit of, the humane use of animals in biomedical research; and to lead a coordinated response to the pressure on the laboratory animal supply chain, and the licence to use laboratory animals in research. It seeks to provide support, advocacy and reliable communication on behalf of public and private researchers at both national and European levels.
Animal research remains a contentious issue with a strong vocal opposition. As a result, public engagement by many European researchers and institutions remains hesitant and often defensive. This lack of positive communication allows the voices of those opposed to animal research to dominate public discourse, with the result that public and political opinion is often uncertain on the use of animals in research. This has the potential to lead to further restrictions on research to the detriment of science, medicine, and society.
For too long, the scientific community (with some noticeable exceptions) has allowed the fear of animal rights extremism to prevent its members from speaking publicly about animal research. Today, this fear, although understandable, is increasingly unfounded. While some researchers may encounter animal rights groups involved in vocal but lawful activities, very few will ever come across extremists. These animal rights groups are often large, well-funded organizations with professional advocates, lobbyists, and media consultants, who can successfully command public discussion on the subject of animal research (often with misinformation and unfounded opinion); particularly in the absence of public communication from the scientific community. As a result, members of the public are rarely exposed to a comprehensive, well-informed, and balanced overview of the subject.
Greater openness on the use of animals in research can encourage public trust and allow the scientific community to speak with a united voice. In doing so it can prevent individuals and organizations from being isolated. Pro-active communications will help to garner support and improve understanding; non-communication will only prolong opposition and mistrust. We all need to play a role in illuminating the complex social issues involved with animal research and its benefits to human and animal health. The scientific community should not allow those who are opposed to animal research to set the public agenda.

OF1M2 GIRCOR - Who are we? Missions and commitments

Balansard, Ivan, Presenting author
GIRCOR

GIRCOR brings together the biological or medical research institutions in France as a non-profit organization: public research institutions, large institutes, pharmaceutical companies and private research institutes take all part. GIRCOR is currently presided by the CNRS
GIRCOR brings together the biological or medical research institutions in France as a non-profit organization: public research institutions, large institutes, pharmaceutical companies and private research institutes take all part. GIRCOR is currently presided by the CNRS. The use of animals for scientific and medical research is widely practiced in the world because it is essential to medical progress. But because of the lack of information, the public poorly understands the reasons and the conditions in which it is practiced. This sometimes results in misunderstandings and misconceptions. GIRCOR and the scientific community address the need for clear and transparent information.
By informing on current research, GIRCOR gives everyone the opportunity to know the place animal research has in scientific and medical progress. Furthermore, it outlines and explains the conditions under which animal research is practiced. Importantly, GIRCOR also acts via Grice – a working group part of GIRCOR that drives the reflection and work of ethics committees in France – to encourage the development and actions of ethics committees within research institutions.
GIRCOR informs on the role of animal research in recent advances in biological and medical research. It also provides information on the development of regulations and techniques. GIRCOR is committed to provide its resources and expertise to service the generation and distribution of fair, precise and comprehensive information based on referenced sources. Visual information (video or infographics) is used whenever possible.
Concerning the ethics committees, Grice wrote in 2009 a reference guide to the ethical evaluation of animal studies (in French and English). Today, Grice pursues its mission and organizes the training for members of the ethic committees.

OF1M3 Research4Life: an Italian platform for science advocacy

Grignaschi, Giuliano, Presenting author
Research4Life

Biomedical research in Italy is undergoing a process of de-legitimization that is causing serious damage to our scientists. False information is giving rise to conspiracy theories that need to be counteracted. The aim of Research4Life is to counter the spread of false information, giving a voice to researchers, patient associations, medical doctors, industry and charities.
Body text (max. 2500 characters, no further requirements regarding the format): Biomedical research in Italy is undergoing a process of de-legitimization that is causing serious damage to our scientists. False information is giving rise to conspiracy theories that need to be counteracted. The aim of Research4Life is to counter the spread of false information by giving a voice to researchers, patient associations, medical doctors, industry and charities. In few months of activity we have grown from 10 to more than 30 partners and gained the trust of competent authorities, media and academia. Particular attention has been devoted to animal testing and to the incorrect transposition of EU Directive 63/2010.

OF1M4 Informing the public on animal research

Tineke Coenen, Wilbert Frieling, Martje Fentener van Vlissingen
SID, The Netherlands

Opponents and activists used to be the main provider of public (dis)information about the use of animals in research and education. The scientific community mostly failed to provide their own information (typically restricted by fear or by institutional PR policies). The Foundation for Information on Animal Research (Stichting Informatie Dierproeven (SID)) was founded in 2004 to fill the information gap and to provide concise, objective and simply worded information on the use of animals in research. SID is supported financially and in kind by a variety of public and private institutions including patient organisations.

The foundation reckons that, presently as well as in the near - foreseeable - future, animal research will be indispensable for fundamental research in the life sciences, biomedicine and human and animal health in general. With these objectives and under proper conditions regarding ethical review and animal welfare, animal research can be done in a responsible and acceptable way. However, the information available to the public (e.g. scientific publications, legislation, annual reports) is not only overwhelming by its quantity (many separate documents) but it is also very complex, too. In short, for an interested lay-person it is virtually impossible to obtain an overview of this field regarding development in the use of animals and possible alternatives. SID aims to fill this gap by publishing fact-based information in a concise way using modern communication tools. The website (www.informatiedierproeven.nl) contains information on and links to a wide variety of topics including a number of interactive components. Questions received by e-mail are answered with the help of an expert available through SIDs professional network. The governmental annual report (statistics) on animal research is transformed in an easy readable version from 2010 onwards. Starting 2012, a special workbook for primary and high-school students was issued. It is interactive, FAQ-oriented, easy to navigate (tool in PDF format) and can be used by individuals preparing a lecture or manuscript, or within a classroom context. It helps students to form their own opinion on this highly relevant topic concerning human-animal interaction. It also contributes to education in the life sciences and biomedicine. This is supported by a school program where scientists voluntarily present and discuss the topic, thus enriching school programs. Lately, the website has been adapted for mobile use, supported by active use of social media. Also short, partly animated films about scientifically new topics are produced challenging people to form their own opinion. In all, the responses from the public are good. The site heads the hit list in internet search machines and is used intensively except during summer holidays, indicating that many young students use it for their school projects.

OF1M5 The Basel Declaration Society, a network of scientists campaigning for the necessity of continued animal experimentation

Zuniga, Aimee, Presenting author
Basel Declaration Society

As in the last years animal right activists campaigns became increasingly active with national governments and the E.U. to tighten laws against animal experimentation and working towards a total ban, scientists have decided to unite to make their voice heard with decision makers.

The Basel Declaration Society (BDS) is an international grassroots organization founded by academic researchers in 2011. The Basel Declaration has been signed by thousands of researchers around the globe that are committed to ethically responsible animal research, the 3Rs and transparent communication about animal research. One of its main tasks is to campaign with all stakeholders including politicians and governments against progressive restrictions and ban of animal experimentation. A second major task is to inform the general public about the importance of animal experimentation in life science research and its benefits for medical progress and improving human health.

The vast network of scientists and BDS ambassadors in different countries allows mobilization of large numbers of supporters in a very efficient and timely manner, which has proven essential at various occasions in different countries. The BDS organizes conferences to allow where scientists, members of the public, politicians, science communicators and patient associations to join efforts in increasing public awareness of the benefits of ethical and responsible animal experimentation for the society as whole.

The international BDS is an opinion leader with both national and international impact. The BDS is seen as a trustworthy partner in those discussions as it relies entirely on voluntary efforts by BDS members and supporters.

OF1M6 Improving public communication on animal research in the UK

Jarrett, Wendy, Presenting author
CEO Understanding Animal Research

How the Concordat on Openness on Animal Research in the UK is helping to improve the quality of public communication and engagement on animal research
In May 2014, 72 organisations published a Concordat on Openness on Animal Research, committing themselves to principles of openness about their work. Two years on, what has changed? This talk will present examples of communications and public engagement and discuss what the next steps may be for the UK bioscience sector.

OF2S1 The ECI on animal research. Can myths shape debate on Directive 2010/63? Example of a European Citizens Initiative

Chlebus, Magda, Presenting author
EFPIA

European Citizen's Initiatives (ECI) are a new European Union's tool to enable citizen's to raise questions and make proposals in areas of perceived legal vacuum or gap. To qualify as an official Citizen's Initiative in the eyes of the European Commission, the petition must be supported by at least one million signatures from across at least eight Member States. So far, three petitions have met these terms and been recognised as a European Citizen's Initiative. These have focused on water, stem cells and vivisection - all topics which are sensitive and complex, and rightly capture the attention of many European citizens.
The 'Stop Vivisection' ECI calls for the abolition of Directive 2010/63/EU which, in the view of petitioners, encourages use of animals in research and testing rather than their phase out. Worth noting, Directive 2010/63/EU provides for one of the most progressive and stringent mandatory animal protection frameworks regarding animal use for research, worldwide. It harmonizes standards across the EU so as to promote both animal welfare and high quality scientific research. It also spells out the EU aspiration to replace, when scientifically possible, the use of animals by other research methods.
This petition is an interesting case study to examine the different communication and advocacy strategies, both by considering the approach of the petitioners (erroneous claims, undermining scientific achievements, over-optimistic representation of scientific and technological opportunities for replacement) and the research community's response.
While the number of signatures shows that research involving animals is an area of interest for many Europeans, its call to abolish Directive 2010/63/EU is at odds with animal protection goals and scientific objectives that require an informed dialogue, collaboration and good practice sharing.

OF2S2 Speaking Research

Leech, Kirk, Presenting author
Executive Director, European Animal Research Association and member of Speaking of Research
Deschamps, Anne[1], Co-Author, Brunt, Michael[1], Co-Author, Holder, Tom[1], Co-Author
[1]Speaking of Research

Scientists and laboratory animal science professionals (LASP) each have a crucial role in educating the general public and policy makers regarding the importance of this work. Scientists are able to provide unique insights about how and why they use animal models. Why is it important? How will animals and humans benefit from the knowledge that is gained? LASP are able to communicate the conditions in which the animals in scientific studies live. How are they cared for? Who looks after them? Are they treated with compassion and respect? Speaking Research (SR) believes that animal research should be conducted with the utmost care, responsibility, and respect towards the animals. Another important avenue is describing the current threats to scientific research. Activist infiltrations of animal facilities often misrepresent conditions in the laboratories they film, significantly undermining public trust and support for scientific research. Animal rights groups have been known to exert pressure on individuals and companies in an effort to stop animal experiments. Recently this activity has moved into the political arena with animal rights groups lobbying for legislative reform or challenging research in courts. Another significant threat to scientific research has been the progressive decrease in funding for fundamental or basic research. At the same time, increasing cost and regulatory burden, sometimes without evidence of meaningful improvement in animal welfare, challenge the conduct of science. All of these threats require proactive discussion between scientists, LASP, policy makers, and the general public. Speaking Research (SR) and the European Animal Research Association (EARA) believes that accurate information is necessary to underpin honest discussion surrounding the role of animals in science.
For too long the only information that the public could access about animal research was provided by organisations opposed to the use of animals in scientific progress. We believe that greater openness with the public on animal research helps dispel the myths provided by those opposed to research. Greater openness should become the norm in all European countries.

OF2S3 What do people working with animals in research think about other uses of animals in society?

Mortell, Norman, Presenting author
Agenda Life Sciences

Over 300 people working within research responded to a survey conducted in 2015, these were individuals working directly with animals to ascertain their views on a variety of uses of animals in society. The objectives were to review whether people working with animals in research care about other animal welfare issues, to review whether these are in line with other public surveys and when recruiting people into the research sector whether there is a "baseline" of animal welfare interests.
People who oppose the use of animals in research often portray those working within the life science sector as lacking compassion and being uncaring about the welfare of the research animals. This survey explores how people working with animals in a variety of roles and within different types of institutions care about animal welfare across a range of ways that animals are used in society. In addition, the research community has historically been suspicious of new job applicants that indicate that they have a keen interest in animal welfare, this survey shows whether people in research have a "baseline" background interest in animal welfare issues and reviews other surveys to compare and contrast the results.

OF3S1 Improving statistical data on animal use in the EU

Louhimies, Susanna, Presenting author
[1]European Commission
Anderson, David, Co-Author
[1]European Commission

Comprehensive and accurate information is the prerequisite for any decision making be it for policy making, research funding or simply to understand the status quo. To improve transparency and availability of information on the use of animals in scientific procedures in the EU, a main objective of Directive 2010/63/EU, the intention was set to fully revise the requirements for statistical reporting.
Commission Implementing Decision 2012/707/EU adopted in 2012 lays out the standard data categories under which future reports at European level will be presented. Reporting in the Member States under the new obligations has already commenced with the first national reports published in 2015. The key changes include the timing of the reporting, the level of detail of information to be recorded and a number of new reporting elements such as actual experienced severity, genetic status of the animal and origin and generation of non-human primates. In addition, future EU reports do not only count animals but each use is counted separately. These changes create two important challenges: on the one hand to ensure the collection of complete and accurate data, each user needs to know what needs to be recorded, what are the basis for the reporting and how the reporting is carried out in practice; on the other, the new type of complex statistical data that will be made available in the future needs to be explained to the general public so that the information drawn from the reports is understood correctly. The Commission together with Member States and user community is working together to provide the necessary tools to make this change a success.

OF3S2 Spanish experience reporting under art.54.2 of Directive 2010/63/EU

Leon, Pilar, Presenting author
MAGRAMA, Spain

Directive 2010/63 / EU of the European Parliament and of the Council of 22 September on the protection of animals used for scientific purposes, provides in Article 54 the obligation to Member States to notify the Commission each year before November 10 statistical information on the use of animals in procedures, and to publish this information.
MAGRAMA IT services designed an application to facilitate the collection, monitoring and data transmission from users Spanish centres using animals to DECLARE. The information is provided by authorized centres, which are introduced the respective reports in the system, and it allows to regional competent authorities to supervise those reports and forwarded them to MAGRAMA, the point to transmit all the communications to the European Commission.

After that, information collected can be grouped by a plenty of combinations of criteria and extracted from DECLARE to build tailored reports.

Last year experience gives the opportunity to learn how to deal with the system, how to improve the quality of the reports by proposing new errors and warnings in the validation tests, and to imagine the wide possibilities of the system in the forthcoming years.

OF3S3 Users experience, good practice and challenges to implement new statistical reporting under Directive 2010/63/EU

Robinson, Sally, Presenting author
AstraZeneca
Chlebus, Magda, Co-Author
EFPIA

To ensure maximum transparency about the use of animals in research and development, but also to adapt the forms to the scope of the revised Directive 2010/63/EU, the statistical reporting templates and rules have been changed.
There are some practical challenges that have to be considered to make sure that the reporting can be accurate and meaningful. It starts with aligned understanding of definitions of projects and procedures, the ability to measure and record and retrospectively report severity, differences of interpretation of what use/continued use/re-use mean in practice. Continued dialogue, information to users and training, are not yet a widespread practice across Europe. Time needed to adjust processes and reporting tools in research establishments should not be underestimated. The presentation will address users experience, good practice and challenges that can be addressed through collaboration.

OF3S4 A review of EU non-technical summaries; good and bad practices

Taylor, Katy, Presenting author
European Coalition to End Animal Experiments

Under the new Directive 2010/63/EC Member States have to ensure that non-technical summaries (NTS) of authorised projects involving animals are published. Article 43 asks that this summary include information on the objectives of the project including the predicted harm and benefits and the number and types of animals to be used. Summaries should also demonstrate compliance with the 3Rs.
The intention was that NTS would provide some increase in the transparency of animal research in each Member State. In 2012 the Commission worked with Member States to provide guidance including a template for the summaries that allows this information to be included (EC 2013). However, although the provisions of the Directive had to be adopted in each Member State by 1 January 2013, there was no specific timescale given by which the NTS had to be published, nor how frequently and how soon after the project had been authorised.
In Jan 2016 the European Coalition to End Animal Experiments (ECEAE), which has members in most EU countries, surveyed all EU member states to identify which ones were publishing their NTS and how frequently these were uploaded. Not all Member States are yet publishing their NTS and some are publishing them with some delay, more than two years in some cases.
In this presentation we summarise the status of publication of NTS across Member States and compare best practices both in terms of publication rate, transparency and accessibility. We also include our observations on the quality of the NTS and the extent to which, within their limited length, they adhere to the requirements of Article 43. We pay particular attention to reporting of 'harms', 'benefits' and the implementation of the 3Rs and we offer suggestions for improvements.

OF3S5 Germany's web-based solution for publishing non-technical summaries

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Bert B, Vietze J, Schönfelder G, Grune B, Co-authors

The Directive 2010/63/EU requires the Member States to publish non-technical project summaries (NTS) on authorized animal experiments. To fulfil this obligation, Germany established a freely accessible and searchable NTS-database.
The obligation to publish the NTS is a crucial instrument on the way to ensure transparency and to disseminate information on animal experiments to the public. As there are no further provisions in the Directive on how the NTS should be published, the Member States can choose their own way, as long as the legal requirements are fulfilled and the goals of the Directive are being implemented appropriately. In Germany, the Federal Institute for Risk Assessment (BfR) is responsible for the publication of the NTS. To facilitate the workflow between applicants, competent authorities and the BfR, and to ensure an easy accessibility for the interested public, a web-based database has been created. This database allows users a search term based retrieval providing a comfortable tool to search for the purpose of experiments, application of the 3Rs, animal species or any other type of information provided in the NTS. This user friendly approach opens new channels to make data on animal experiments fully transparent and accessible. Moreover, the database offers the great opportunity to analyse the data provided by the NTS and to detect those domains for animal testing where research for alternative methods is urgently required. Thus, the database does not only enhance transparency, but also contributes to promote animal welfare in the future.

OF4S1 Report from the AALAS - FELASA working group on harm-benefit analysis of animal studies

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International regulations and guidance strongly suggest that the use of animal in research should be initiated only after the responsible for the review has concluded a harm-benefit analysis (HBA). In many institutions the relevant factors and algorithms used in conducting the HBA are poorly defined or lacking. The aim of the AALAS - FELASA working group on HBA was to define the concept of harm-benefit analysis and recommend how it can be implemented.
Methods: Literature review included papers from 1986 to 2015. References on cost/risk benefit from other industries were also included. Results Several approaches to HBA were identified including algorithms, graphic presentations and generic processes. Strengths and weaknesses of different HBA-methods were analysed. The common definition of harm was based on a several factors mainly influencing animal welfare, but also factors of the animal rights domain were identified. The 5 freedoms were suggested as a foundation for harm assessment. Severity categories for harm are also defined. Benefit domains include benefits for humans, animals, environment, knowledge and education. It was questioned if economic benefits alone can justify animal use. Subjective opinions cause problematic bias in HBA. A synthesis of the different methods for HBA previously describes was used by the WG to create a new tool for HBA. To limit bias different modulating factors with aggravating and mitigating effects were defined. A colour scale was used to give an intuitive, visual result of the final impact of harms and benefits - taking mitigating and aggravating factors into consideration - to give a better overall impression of the HBA without the misleading precision of an arithmetic assessment. The tool has been used in several work-shops on HBA. Examples of the working groups approach illustrations are included in the 2 working group reports. Conclusion We define HBA as a systematic, transparent way to define harms, benefits and how they are balanced. We base the harm-domain on factors that impact animal welfare. The WG has summarized the contemporary understanding of harm and benefit. Several approaches to HBA were already suggested. The WG proposes a practical methodology to address HBA analysis that we consider as a refined approach compared to previous suggestions.

OF4S2 Promoting consistency in Project Evaluation under Directive 2010/63/EU

Anderson, David, Presenting Author
Technical Advisor to European Commission
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European Commission

The Project Evaluation process is a main cornerstone of Directive 2010/63, requiring that projects may only be authorised following a favourable Project Evaluation which determines, in addition to meeting the legal requirements of the Directive with regard to compliance with various articles, including the implementation of the Three Rs, that the level of harms to be experienced by the animals is justified by the expected outcomes. The process needs expertise in a number of areas, and shall be impartial, subject to safeguarding intellectual property and confidential information, and transparent. Based on the outcome of an Expert Working Group, a guidance document, to assist Member States and others affected by the Directive is available at the EC website http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm. A further EWG was convened at the request of MS to develop a few examples of the evaluation process. This work has clearly demonstrated that although the outcomes may be similar, the information required varies significantly, reflecting the differing structures for Project Evaluation developed within MS.

OF4S3 ANIMPACT: Mapping European diversity under Directive 2010/63/EU and proposing tools to increase dialogue

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Greater harmonization in terms of uniform regulation of research with animals, including uniform animal welfare standards, was an important objective in revising European legislation. Technical harmonization is facilitated by the revised and expanded Directive, but Member States (MS) still have flexibility in establishing mechanisms under the Directive. The ANIMPACT project is mapping ethical and practical aspects of the legal framework, with focus on decision-making over animal experiments.
The ANIMPACT project addresses how Directive 2010/63/EU interacts with research by looking at decision-making mechanisms. These include regulatory mechanisms external to research (legal norms and the licensing process, ethical norms and the ethical review process), mechanisms internal to research (how the 3Rs are considered in peer review, how researchers select animal species) and the intersection of the two (how researchers perceive regulation and how their work is impacted by it). The emerging results show considerable diversity, in particular in systems for project evaluation and authorization of animal experiments. The aspects that the project evaluation is to include (e. g. predicted benefit, 3Rs compliance, severity, harm-benefit analysis) are established by the Directive and subsequent endorsed guidelines. However, there is a lot of room for interpretation of key concepts such as "predicted benefit" and "harm-benefit analysis". Furthermore where and by whom projects are evaluated is left to individual MS to determine. The result is a wide variation in approaches. Depending on the MS, a project may be evaluated by a national, regional or institutional committee, by people with varying scientific and other expertise, with or without the involvement of lay members / special interest groups. To this procedural diversity should be added the diversity in outcome expected because of the mentioned room left for interpretation left in the hands of differently composed groups of people. Plous and Herzog (2001) found that institutional animal care and use committees in the US (thus in the same country and under the same guidance) took widely different decisions over the same animal protocol. The total number of committees in the EU is not known, but considering that at least 15 Member States have regional, local and / or in-

stitutional committees, they are expected to be several hundred. More than detailed guidance, we argue that these committees need mechanisms for dialogue in order to avoid a situation where similar protocols are evaluated very differently by different committees. One such approach may be to regularly publish commented case studies, such as the Protocol Review column in the journal Lab Animal. We are presently exploring ways of establishing a European equivalent. At the conference we will also invite an open discussion of potential formats and mechanisms to create discussion fora for entities involved in project evaluation across the EU.

OF4S4 30 years of experience in the ethical evaluation of animal experiments: a new era

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Like many European countries, the Netherlands has a tradition of ethical review of animal experiments. Already in the 1980s the first Animal Ethics Committees (AEC) have been established and since 1990s ethical assessment has become compulsory. As a result, the Netherlands has over 30 years of experience in ethically evaluating animal research. The question, however, is this experience helpful in the new situation in which the 2010/63/EU directive has been implemented.
At this moment 18 Animal Ethics Committees in the Netherlands advice a single Competent Authority: the Central Authority for Scientific Procedures on Animals (CASPA). Before the AECs advised the license holder they were (mostly) affiliated to, and in most cases a positive advice by the AEC entailed an approval to perform an experiment. Now CASPA grants licenses for animal experiments based on the advice of the AEC. This implies that the individual committees can no longer work independently as they did. In this process of exchanging knowledge and best practices in a changing field, the Dutch Society of AECs aims to play a supporting, yet central role. This organisation functions as a platform for information exchange and discussion amongst the AECs.
The general legal attitude towards animal testing remain unchanged. The "no, unless-principle is still the base of the law and the necessity of an ethical evaluation remain unchanged. However there are some real changes in the process of making assessments.
First, individual experiments were the object of the assessment now under the new law, the ethical evaluation focusses on full projects. This requires that the ethical evaluation includes discussions on basic assumptions and principles, and less on technical details. This has direct consequences.
Furthermore, we start the ethical evaluation only if the discomfort equals or exceeds that of introduction of a needle. Secondly, the performance of procedures that result in severe pain, suffering or distress, which is likely to be long-lasting and cannot be ameliorated should be prohibited.
Finally, next to the non-technical summery that is written for the general public, the Dutch Freedom of information Act enables citizens to get access to everything in the procedure, including the written ethical advice. This makes both the CASPA and the AEC's publically accountable. Transparency and putting the evaluation in writing has proven to be a real challenge. Especially to set the necessary mutual understanding with CASPA. To conclude, the experience in the Netherlands has benefits for the current situations. Best practice has been defined and lessons have been learnt. Nonetheless, the current transition asks for cooperation between the AEC's and the CASPA. The Dutch example shows that a platform of AECs can actively offer knowledge, and help to bring experience and skills of the ethical evaluation between AECs together. Thereby contributing to the new partnership and facilitating transparency.

OF5M1 Rehoming of laboratory animals – analysing animal welfare pitfalls and presenting possibilities

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Belgium has implemented Directive 2010/63/EU in its national legislation in 2013, stipulating that an ethical review body can release a laboratory animal for return to its natural habitat or to a husbandry system suitable for the species, or for rehoming. In order to make this decision, the health and welfare of the laboratory animal, as well as the safety of the public and the environment must be considered. In case of rehoming, an adoption programme must be in place that foresees in the 'socialization' of the animal.
In this paper, we critically review the rehoming of laboratory animals, by pointing out potential threats to animal welfare and discussing the laboratory animal rehoming programme of the Faculty of Veterinary Medicine at Ghent University, which was developed and approved in 2014 and has been running since January 2015.
From a welfare point of view, there are several issues to consider when rehoming former laboratory animals. First, the risk of adjustment problems is greatest when significant discrepancy in the social and non-social environment exists between laboratory setting and environment post rehoming, like when laboratory animals are rehomed as companion animals. This risk involves not only the welfare of the laboratory animal, but also that of humans and other animals in the new environment. Next, the expectations of the new owner towards the animal may be greater than its coping ability, resulting in additional stress for the animal and disappointment in the owner. Finally, when animals have a chronic ailment, due to old age or other, the adoption must be considered carefully in light of the required treatment to maintain sufficient quality of life for the animal.
The laboratory animal rehoming programme of the Faculty of Veterinary Medicine at Ghent University consists of screening of laboratory animals when available for adoption, screening of potential adopters by investigating the match with a particular animal in terms of housing facilities and adopter expectations, informing potential adopters about the social and medical background of the animals, and the signing of a rehoming contract. Because dogs and cats live in close contact with humans (adults and children) and possibly other animals, the above-mentioned welfare risks are even greater. An additional step in the adoption programme for these species, therefore, involves observation of the animal in different contexts to assess the behavioural strategies an animal is likely to adopt post rehoming, and a meeting with potential adopters by an animal behaviour specialist. During this meeting, particular attention is paid to educating potential adopters about canine or feline stress signalling, and to motivate them to use this knowledge to facilitate the adjustment process.

OF6M1 The Animal Welfare Body and its tasks

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Directive 2010/63/EU requires that each establishment that breeds, supplies or uses animals has an Animal Welfare Body (AWB) to provide advice on the welfare and care of animals.
Directive 2010/63/EU states that animal welfare considerations should be given the highest priority in the context of animal keeping, breeding and us for scientific purposes. One of the mechanisms within the Directive to achieve this aim is the creation of an Animal Welfare Body in each establishment. Articles 26 and 27 of Directive 2010/63/EU set out the composition and requirements for Animal Welfare Bodies. The tasks include advising staff on the welfare of animals, the application of the Three Rs, advising on rehoming schemes and following the development and outcome of projects. An effective Animal Welfare Body has many benefits for both animals and science and for the staff working with animals, and is a key contributor to fostering a good culture of care within the establishment.

OF6M3 'Jump-starting' the implementation of Animal Welfare Bodies in Portugal: a challenge for all stakeholders

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EU legislation demands an Animal Welfare Body (AWB) to be in place in establishments where animals are used. With no previous tradition of AWB (or AW officers) in Portugal, with few institutes previously having animal ethics committees (which were not legally required), and with little guidance from the competent authority, setting up AWBs poses significant challenges to several institutes and universities. We present our current efforts to help "jump-start" AWBs, and point future directions.
The 2010/63/EU [1] brought significant changes to how animal research is evaluated, supervised and reported to regulators, particularly to countries with previously less stringent legislation, such as Portugal. It is however an opportunity for raising standards of competence and functionality in planning, reviewing, and supervising procedures within institutions, while promoting a culture of care. The Portuguese transposition [2] gives AWBs an influence beyond what is established in the Directive, including a central role in project evaluation, with the responsibility to issue non-binding appraisals for the Competent Authority (CA).

To promote dialogue and facilitate information dissemination, in 2014 two workshops were held with existing and future AWB members in Porto and Lisbon, with the support of the Portuguese Laboratory Animal Science Association (SPCAL) and the CA. These comprised three themes – "Establishing an AWB", "Running an AWB", "Setting-up an AWB Network" – and lectures by: a representative of the CA, two designated vets, three LAS researchers (and SPCAL members) and the director for the 3Rs of a large pharma company. Key issues concerning governance and regulatory compliance (with particular focus on project evaluation and severity assessment) in animal research were covered.
Subsequent to the workshops, we carried out an on-line survey from July to September 2015, sent to the heads of already working AWBs or, alternatively, of animal facilities of Portuguese research institutions. Of the 25 institutes identified as having research with laboratory animals and with contact details available, 22 replied. The survey aimed at assessing the status and composition of AWBs, the main challenges faced, and whether there was an interest in establishing a national network of AWBs. Of the 22, only eight had by September 2015 an AWB up and running, an equal number were in the process of establishing one and the remaining six had not yet began this process. Interest in a national AWB network was however high, with 17/22 respondents considering it "very important" and declaring themselves willing to participate in starting it up. [3]

We are now moving forward in 2016 with starting an AWB network, with the support of SPCAL, along with organizing a series of workshops on severity assessment and statistical reporting across the country, as well as a first symposium for AWB members. We welcome contacts with other national and European-wide networks for AWBs.

OF6M4 Aware of quality: risk based quality assurance in the academic setting

Verhave, Peternella Suzanne, Presenting author
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The Experiments on Animals Act in the Netherlands requires that the Animal Welfare Body performs post approval monitoring (PAM) next to the fine tuning animal experiments before the start. The Animal Welfare Body Utrecht (AWB Utrecht) described these processes in a quality plan. The primary goal of the quality plan is to structurally approach PAM of experimental animal work at the University Medical Centre Utrecht and the Utrecht University.
The quality plan is intended to ensure animal well-being while at the same time guaranteeing that all animal experiments are in accordance with legislation. Quality assurance is a team effort and can only work if there is quality awareness among staff. We aim to give all certified personnel insight in the wide range of rules and regulations. We do this by broad internal communication of the quality plan within the facilities. In addition, the AWB Utrecht pays attention to the 3R's, new insights in animal experimentation, administration and procedures and also specifically on animal welfare in a monthly newsletter. The AWB Utrecht aims to communicate comprehensibly with certified personnel on site by communicating in Dutch and in English.
An important tool for creating awareness on quality is structural visibility of the practical implementation of the quality plan at the workplace. Audits are performed by highly skilled animal welfare officers and veterinarians. Of similar importance are the regular peer monitoring and 'self'-monitoring tools used in the quality plan. The different monitoring techniques which are part of the quality plan help departments, animal facilities and researchers to focus on the critical steps in the conduct of animal experiments. It also assures regular communication about quality between AWB members and all personnel involved.
Risk based audit planning is described in the quality plan and is the base of the PAM as performed by the professionals of the AWB Utrecht. Departments are ordered by number of animal experiments and average discomfort levels on a yearly basis. In the future, audit findings and competence will be included in the risk based audit planning. A systematic approach to communicate about quality assurance and awareness ensures compliance and animal welfare to stay high on the agenda. With an emphasis on quality throughout the chain, the AWB Utrecht stimulates and assures the responsible use of laboratory animals.

OF6M5 Implementing the AWB in the UK - developing a culture of care with the Animal Welfare and Ethical Review Body (AWERB)

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The UK has implemented the requirement within Directive 2010/63/EU to set up an Animal Welfare Body (AWB) by forming and maintaining Animal Welfare and Ethical Review Bodies (AWERBs) at every breeding, supplying and user establishment. These have additional roles, and wider membership, than the minimum requirements for the AWB listed in Articles 26 and 27.
When the UK implemented Directive 2010/63/EU, all breeding, supplying and user establishment already had a local Ethical Review Process (ERP) in place, which was well established and valued, providing a good model for the AWB. The revised UK Animals (Scientific Procedures) Act 1986 (ASPA) therefore includes a requirement for each establishment to set up an Animal Welfare and Ethical Review Body (AWERB) which carries out the five minimum tasks of the AWB, as set out in Directive Article 27 [1]. The AWERB also has additional advisory and reviewing tasks:

- advising the establishment licence holder whether to support project proposals, primarily considering such proposals from a local perspective and bringing to bear local knowledge and expertise;
- assisting with the retrospective assessment of relevant projects; and
- responding to enquiries, and considering advice received, from the National Committee (the Animals in Science Committee).

The UK regulator (the Home Office) emphasised that the AWERBs 'should in most respects continue and develop the work of the local Ethical Review Processes they replaced on 1 January 2013', and this is reflected in the general requirements of the AWERB to promote awareness of animal welfare and the Three Rs; provide a forum for discussion and development of ethical advice; support the personnel with specific requirements listed in Directive Article 24 on animal welfare, ethical issues and provision of training; and promote a culture of care [2]. This presentation will set out an overview of the operation of the UK AWERB from an independent member's perspective, including what has worked well, tasks that need more attention, useful resources, and how the AWERBs and National Committee interact.

OF7S1 Drivers and Barriers for the implementation of 3Rs Principle. An industry perspective

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GSK Vaccines
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The principle of the 3Rs — Replacement, Reduction and Refinement - is widely accepted internationally as criteria for humane animal use in research and testing. Its application requires the contribution of multidisciplinary areas of expertise: laboratory animal science, basic biology, assays' development, pharmacology and toxicology, regulatory practices, as well as ethics and behavioural sciences
The principle of the 3Rs — Replacement, Reduction and Refinement - is widely accepted internationally as criteria for humane animal use in research and testing. Its application requires the contribution of multidisciplinary areas of expertise: laboratory animal science, basic biology, assays' development, pharmacology and toxicology, regulatory practices, as well as ethics and behavioural sciences. Taking into consideration animal welfare, economic and scientific perspectives as well as international horizontal legislations to protect animals used for scientific purposes, The demand for implementation of 3Rs philosophy in testing of human life saving products is on the increase and it is coming from many actors in our society: general public who is more sensitive on the ethics of animal research; legislators; public and private research institution who recognized that the best science is the one which cares about animal welfare if animals are still required to advance human kind knowledge. The presentation would like to highlight the vaccine manufacturing industry's commitment and its concrete programs for the application of the 3Rs principle in their R&D, manufacturing and quality processes; its external collaborations with other industries and public institution for the global acceptance of alternative methods. However, the presentation would like to reveal the internal and external barriers to the application of the 3Rs principle and share its vision on how to overcome them.

OF7S2 An industry approach to animal welfare – Novo Nordisk bioethical set-up is now also including a dedicated 3R Department to ensure a strategic approach

Øvlisen, Kirstine, Presenting author
Novo Nordisk A/S

At Novo Nordisk it is of utmost importance to carefully consider ethical implications of our activities when developing treatments. At present animals research is essential in all pharma companies and we recognize that it cannot be completely replaced in the foreseeable future. We therefore strive to ensure a responsible approach by setting high global standards in our housing and care of animals. To ensure continuous improvements within the 3Rs a dedicated department has been established.
Attitudes towards animals vary with national perceptions and regulation. Novo Nordisk has therefore established a set of principles and global standards for the housing and care of animals, reflected in a series of Standard Operating Procedures and internal guidelines. The principles follow the latest and most comprehensive international guidelines (ETS No. 123, Appendix A, Directive 2010/63/EU), which regulates the use of animals in research and takes the physiological and behavioural needs of the animal species into account. Furthermore we follow the local laws applicable at our different sites and view the regulations that set the highest bar as the minimum requirements. Novo Nordisk's bioethical work is governed by the Social and Environmental Committee, mandated and chaired by Novo Nordisk's Executive Management. The Committee is responsible for the company's policy and strategy on bioethics. To strengthen the focus and ensure continuous improvements a comprehensive governance system for activities related to the use of animals in research has been established. A cross functional team of approximately 70 employees work to ensure that Novo Nordisk's business activities are ethically sound and that adherence to the principles are found throughout the company. Novo Nordisk's major inherent bioethical issues, one of which is animal research, are addressed by expert groups. Expert groups ensure that emerging issues are addressed. A Bioethics Council strengthens the coordination of bioethical activities and ensures implementation. An Ethical Review Council ensures that all research involving animals, at Novo Nordisk or on behalf of the company by external collaborators are reviewed and approved prior to initiation. To ensure that standards are global and truly permeate our organisation worldwide, the global responsibility for animal welfare and ethics are anchored with the Vice President of Laboratory Animal Science in headquarters. Within this function area a 3R Management & Strategy department has been established. This department must ensure a strategic approach to 3R innovation and an even

deeper integration of 3R considerations. The department must ensure internal awareness and education as well as knowledge sharing. Specific focus is given to identify replacement opportunities. Furthermore, the department will engage in dialogue with our key stakeholders and enter into collaborations and partnerships. A dedicated team of five comprise the department.

OF7S3 Animal Welfare Bodies and IACUC – One International Laboratory Animal Breeder's Approach to Common Oversight Challenges across a Distinct Regulatory Landscape

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Taconic Biosciences GmbH
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As an internationally operating organization we are facing diverse requirements for oversight functions which are required by the European Directive 2010/63/EU and its adopted national laws, by US legislation and by accreditation programs. Here we report and discuss our approaches of implementing several oversight functions and with a focus on one European site, we highlight its improvements in the last five years as the landscape shifted from one of voluntary conformance to enhanced compliance.
Originally each of our sites had its separately managed appropriate oversight functions depending on the local regulatory requirements. In the US, Institutional Animal Care and Use Committees (IACUCs) are appointed to review and approve animal care and use programs. By Directive 2010/63/EU institutional oversight functions are defined for an Animal Welfare Body (AWB) and the Animal Welfare Officer (AWO). As additional guideline the Guide for the Care and Use of Laboratory Animals (the Guide) is used for program evaluations by accreditation organizations. Prior to 2010 at our site in Germany it was solely the AWO having internal oversight function. With the preparation for the first accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, an institutional Oversight Body (OB) was claimed. We followed the Guide regarding composition and responsibilities. This OB has an advisory role in matters related to animal welfare and care and use, it is establishing and reviewing internal processes and conditions and offers statements. For sustained observation we implemented semi-annual facility and program reviews. We achieved an effective oversight organ such that no severe or non-compliance incidents were identified by our authorities or auditing organizations within the last years. Additionally the exchange of key activities with OBs from other sites is of great value. With the adoption of Directive 2010/63/EU into national German law in July 2013 an AWB became legal requirement, which we resolved by establishing the AWB as second oversight body. Our AWB is composed according the Directive and focused on legal issues and scientific protocol discussions. The OB was continued, as it successfully addresses more internal issues, discussed directly with the animal care staff. Both bodies run in parallel and overlapping members guarantee the cooperation. Due to the different accessibility and educational background of the body members we thus take advantage of the full potential of animal care. With this presentation we want to show an example for combining established structures with new requirements. We are convinced that the cooperation of several Oversight Bodies and the international exchange is of great value for taking out the best pieces of each program. We want to encourage others to consider how oversight can become a valuable tool in reaching more than the legal requirements.

OF7S4 The importance of global standards and principles to ensure high animal welfare and good collaboration when working with external partners

Juhl Hansen, Jesper, Presenting author
Novo Nordisk A/S

Approx. 20% of Novo Nordisk animal studies are performed at external business partners all over the world. Experience has shown that attitude towards animal welfare and ethical issues vary with national perceptions and regulation. Animal welfare and ethical considerations do not have borders and are given a high priority in Novo Nordisk. To address this challenge Novo Nordisk has established a set of principles and global standards for the use, housing and care of animal in research experiments.
Before initiating collaboration with external partners involving animals at Novo Nordisk both the animal facility at the external collaborator and the external study protocol needs to be approved. Studies cannot be initiated without approval. If approval fails in one of the two steps, a dialogue is initiated to investigate if collaboration can be made possible. The first step is to obtain approval of the external collaborator prior to initiation of a project. This is done by an "on-site" animal welfare monitoring investigating compliance with Novo Nordisk global standard and principles which are based on ETS No. 123, Appendix A. All deviations from Novo Nordisk global standard will be described and evaluated during the animal welfare monitoring. This normally starts a constructive dialogue between animal welfare monitor and the external collaborator on how animals involved in Novo Nordisk studies have to be housed. In the end an "Animal welfare Statement" is signed by both parties stating that the collaborator will apply to "Novo Nordisk principles on the use of animals" and comply with Novo Nordisk global standard for housing and care. The second approval step is an internal ethical review of the external study protocol done by Novo Nordisk Ethical Review Council (ERC). ERC members cover different areas of expertise as animal care and ethics, legislation, study design, 3R initiatives and statistics. The review has focus on adherence to Danish/European legislation, Novo Nordisk and 3Rs principles, and humane endpoints, pain and distress etc. This step also provides a good opportunity for a strong dialogue between the external collaborator, the internal scientist and ERC. Even though decisions made in both of these two approval steps may increases cost and/or prolong the initiation of studies, Novo Nordisk believes that improved animal welfare will lead to more reliable scientific results. Our global standard and principles on use of animals which are the backbone of our approval process of external contractors have resulted in strong collaboration with external partners leading to constant improvements in animal welfare, disseminating and harvesting the values of the 3Rs for all partners.

OF8S1 Regulating the scientific use of wild, feral and free-living animals in the UK under Directive 2010/63/EU

Willoughby, Kim, Presenting author
Home Office, UK
Gray, Peter, Author, Garrod, Kate, Co-Author
Home Office, UK

Directive 2010/63/EU (the Directive) provides specific protection to wild, feral and endangered animals. Advice has been prepared by the UK competent authority for scientists and others working with such animals (excluding wild caught non-human primates) in order to clarify how the Directive requirements will be implemented, to consolidate previous operational policy in this area and to explain when other legislation may need to be considered.
The transposition of the Directive into the Animals (Scientific Procedures) Act 1986 (ASPA) has changed, in some respects, the regulation of wild caught protected animals for scientific or educational purposes in the UK. The Directive specifies that competent authorities can only allow the use of certain types of animal; endangered species (Article 7), animals taken from the wild (Article 9) and stray and feral animals (Article 11), in specific situations. In addition there are restrictions relating to capture, the competence of those capturing the animals and the care that needs to be given to captured animals both before their use in procedures (Article 9) and before they are set free to the wild at the end of their use in procedures (Article 19). Other Articles that have particular significance in relation to the use of wild-caught animals include Article 38, which requires projects to be designed to enable procedures to be carried out in the most environmentally sensitive manner, and requirements relating to the recording of actual severity of procedures (e.g. Article 54). The Home Office is the competent authority in the UK which regulates scientific use of animals under ASPA and has recently prepared an Advice Note on Working with Wild Animals (AN), based on the Directive and how it has been transposed into ASPA. The Advice Note was developed following consultation with stakeholders, including scientists, animal welfare groups, and other regulators. It provides advice for scientists and others working with such animals when preparing a project application to work with these specific types of animals. The main sections include:(i) definitions of the terms 'endangered, stray, feral and wild-caught',(ii) capture, including information on the assessment of competent people, frequency of checking traps, and the unintended consequences of capture such as environmental damage(iii) wild-caught animals used at licensed establishments(iv) use of wild caught animals in the wild(v) methods of identification and the use of externally applied tracking devices(vi) the fate of animals at the end of their use in procedures.(vii) the roles of people with responsibilities under Article 14.We have also provided a range of sources of information and advice.

OF8S2 Wildlife research in practice

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Performing experiments on animals in their natural environment is different from laboratory experiments in so many ways, that field workers seem to live in a different world.
Unlike most laboratory animals, animals in the wild live in a very seasonal environment. This is reflected in their behaviour and physiology. In consequence, one can do most research procedures only at one specific moment in the year. Wild animals can always be lost to the study in case they lose their marking, or do not return to the same area. Animal studies in the wild are nearly always embedded in a series of activities that do not qualify as regulated animal procedures, but that provide essential complimentary data to the research. One of the more curious aspects is that very often most discomfort occurs during the initial catching, but it depends on what happens subsequently whether or not it is to be classified as a procedure. This is true for most studies on mammals, fish, reptiles and birds.
These aspects will be illustrated with two examples. The first is on equipping birds with (satellite-) transmitters and/or GPS-loggers to document their migratory pathways. This used to be typically of pure scientific interest and useful for better wildlife management. However, the advent of avian influenza made this activity suddenly very relevant for society (animal and public health) as well. Due to technical developments, devices are getting ever smaller and can be put on smaller species, while at the same time they work longer and longer thanks to solar cells. Research into other avian-borne pathogens, such as West Nile Virus, is only beginning.
The second example comes from studies on avian personalities, where many different types of experiments are combined, ranging from bringing individuals from the field to the lab for behavioural testing and the bring them back to their territories within 24 hours, to making selection lines for bold and shy individuals. The whole concept of animal personality has given us a handle on individual variation in behaviour, in part based on behavioural genetics that provide for the necessary variation for species survival in case circumstances change. Knowing how an individual behaves in one context allows us to predict how this same individual will behave in a different context.
The rules for animal use for research were obviously written with laboratory studies in mind. Applying them also to studies in the wild is possible, but often not logical nor very easy. Nevertheless applying an ethical evaluation of the balance between discomfort and gain in knowledge is a good thing.

OF9W1 Experiences of openness: how the Concordat on Openness works in the UK

Williams, Bella, Presenting author
Understanding Animal Research, UAR.org.uk

In the UK, the controversies on the use of animals in research had escalated to unacceptable levels of violence and similarly, to defensive measures like strict security and secrecy. Part of the solution was the Concordat on Openness (2014), developed by 40 organizations and now signed by almost 100 organizations: universities, charities, commercial companies, research councils, umbrella bodies and learned societies, all committed to help the public understand more about animal research.
The Concordat on Openness in the UK has proved a great success, and has led strategic change within the research sector. An aim of the Concordat is to shift the conversation about the use of animals in research from a polarised debate about whether research should take place, to a more nuanced discussion about how research is conducted. The Concordat now has almost 100 signatories, all of whom conduct, fund or directly support animal research in the UK. The signatories contribute to the collective flow of information through the UAR publishing channels but also take their own actions to inform and involve the public and media, including social media. Some can even accommodate tours for visitors. This network also provides for exchange on often rewarding experiences and ways to enhance content and means of communication suitable for both the institution and the differential target groups. The information on animal use also caters for science education for a wide audience and sharing expectations on the advancement of medicine and other fields of life sciences research.

OF9W2 Critics welcome! Public communication on animal experiments and 3Rs

Blom, Harry, Presenting author
Utrecht University

Utrecht University and University Medical Centre Utrecht are committed to the Dutch Animal Experiments Openness Code (2008), signed by the Association of Universities in the Netherlands (VSNU), the Netherlands Federation of University Medical Centres (NFU), and the Royal Netherlands Society of Arts and Sciences (KNAW).
Both university and hospital communicate openly about animal experiments and the pursuit for 3R-alternatives with media, NGO's, and the public, in close cooperation with the 3Rs-Centre Utrecht Life Sciences and the Animal Welfare Body Utrecht. The joint organizations see it as their obligation to communicate about animal experiments and 3R alternatives conducted in their facilities (why, how, how many, etc.), their policy, and their view on animal experiments in general. All communication on these topics is based on the core values Mutual Respect, Trust, and Transparency. As partners in communication, these organizations manage their joint ways of external communication by making protocols for several situations. On a regular basis they actively invite NGO's to come and have a look, ask questions, and discuss their concerns. All three partners also welcome requests for animal facility visits, unless there should be compelling reasons not to, which have not occurred since the code went into effect. The organizations can be found on several social media, which are monitored on a daily basis. On the organizations' websites, researchers and animal workers as well as the general public can find news items, internal policy documents, a quality plan, forms, guidelines, joint Animal Experiment Annual Reports, et cetera. At the same time there are necessary restrictions to openness, to protect the privacy of individual employees. Visiting groups or individuals are not allowed to film or take pictures, but can request for any picture they need. Press and television crews are welcome for interviews, but are not allowed to take shots of data, cage labels or persons other than our spokesperson. In this way the joint organizations contribute to the public debate on animal experiments, and at the same time create a safe work environment for employees.

OF10W1 How Facility Management can assure compliance and Governance helping you to sleep easier at night?

Sanderson M, Mortell N, Sanders P, Presenting authors
Agenda Lifescience Europe

There are many ways to operate a research facility, but all facilities have the same key issues to consider, for example, facilitating the research and getting the most out of fixed space and resources. This must also be balanced against the health and safety, animal welfare, legal and regulatory requirements.
Outsourced facility management assists in this regard by ensuring that there are sufficient well trained staff to cover all the facilities requirements. Outsourced facility management is a cost effective flexible resource to help you sleep easier at night.
Topics covered in a fun and interactive workshop

- How a managed service assists in the meeting of Governance requirements?
- Implementation of EU Directive compliant training systems under an FM contract to meet those requirements
- How Facility Management can assure compliance and Governance helping you to sleep easier at night.
- An explanation of quality based assessment systems such as monthly reviews that provide establishments with confidence that Governance needs are being met (including information on some AAALAC process/audit information)
- Formation of Animal welfare bodies
- Competence management/ education and training/ mobility of professionals
- Exploring the importance of performance measurements, regulatory compliance and key performance indicators.

OF11M1 Culture of Care – from words to action

Louhimies, Susanna, Presenting author
European Commission

A good culture of care within an establishment is a prerequisite for the revised EU and national legislation to deliver the anticipated improvements in welfare, use and care practices.
Recital 31 of Directive 2010/63/EU sets the legal anchor to foster a culture of care, to ensure appropriate animal welfare care and use practices are maintained at all times. The responsibility and foundation for a good culture of care rests with everyone dealing with animals bred or used for scientific purposes. Implementing and maintaining a good culture of care in the day to day work requires effective processes to be in place. However, an appropriate culture can only be achieved if the appropriate tools and techniques are made available.EU guidance developed to facilitate the implementation of Directive 2010/63/EU approaches the culture of care from various angles. The different roles under the Directive can all contribute to a good culture of care through actions and activities that are relevant to their work. The guidance discusses culture of care in the context of the roles and activities of Animal Welfare Bodies and National Committees, as well as within the EU Education and Training Framework. Efforts to promote a good culture of care within an establishment should be further supported by an effective inspection system within which the inspectors view themselves as promoters of good culture of care. Good culture of care has its anchor in legislation but goes beyond just compliance. It is a shared responsibility at all levels of care and use practices – it is the time to shift the focus from words to action.

OF11M2 Understanding the needs to develop and implement a culture of care

Reid, Kirsty, Presenting author
Eurogroup for Animals

Establishing, promoting and maintaining a good "culture of care" is a fundamental requirement if legal, ethical and animal welfare obligations, along with wider responsibilities towards employees and the public, are to be met. Evidence that the "right" culture is in place is through a combination of good and focused management communication 3Rs and animal welfare.
Ultimately, to achieve high standards of animal welfare and science, a wide range of factors have to come together to provide the right framework within an organisation. Having the right attitudes, values and people, with everyone engaged and positively contributing, knowing what is required of them and doing the right thing without prompting! This presentation will focus on the key conclusions of a survey carried out looking at the interpretations and concepts taken up within the pharma industry sector, animal welfare and by laboratory animal veterinarians. Respondents to a questionnaire provided their views on culture of care, how they implement it daily and where they saw obstacles in their way from going further. Within industry, the responses of an organisation were received from management level, the scientists and the technicians. Views vary

in defining the concept and the extent it is taken up and what obstacles stand in the way at various levels within an organisation however there are some interesting alignments and commitments. The stand out issues were the need for the right attitude, ensuring the concept was not lot lip-service but actually implemented, the need for there be a commitment to continually improve and to constantly challenge the way things are done as everyone involved has their responsibilities. There is a need for education at an early stage in the career on alternatives and there is a need to be Transparent - Involve animal welfare advocates.

OF11M3 How to measure Culture of Care as in indicator of animal welfare. Measuring Culture of Care by surrogate markers can identify areas for improvements in relation to the principles of replacement, reduction and refinement

Bertelsen, Thomas, Presenting author
Novo Nordisk

Culture of Care consists of many elements of different nature and consequently there is no simple answer or one single definition of this. Eurogroup for Animals' survey, presented by K. Reid at the EUSAAT conference in 2015, gave a wide variety of statements depending on the type of establishment and the type of professional asked describe a measuring tool embracing individual differences and draws a collective and comprehensive picture of attitudes and practices in relation to animal welfare
With the description and explanation of this measuring tool advice and suggestions on how to tailor a survey that measures the Culture of Care in an individual establishment is given. Many establishments using laboratory animals proclaim to have a Culture of Care but it is rarely explained what it is composed of or how it is reflected in attitudes and procedures entailing the use of animals. As Culture of Care consists of many elements you will need surrogate markers that each tie into the essentials or the key values of a Culture of Care and collectively describe features of an organisation, its networks, norms or trust, which facilitate coordination and collaboration towards a continuous improvements of animal welfare. The model for measuring Culture of Care is based by the principles of the concept of Social Capital which deals with building and deploying strong relations within an organisation, improving quality and efficiency alongside a healthy psycho-social working environment. Some statements from establishments align a Culture of Care with compliance to minimal legal requirements. In our opinion a Culture of Care clearly goes beyond this Culture of Compliance as it also comprises a Culture of Challenge. Measuring the Culture of Care can identify areas for improvements and facilitate the practical and timely implementation of developments in relation to the principles of the 3 Rs. Three main, top-level values – collaboration, trust and integrity – are the 3 essentials for the underlying surrogate markers. Collaboration - means that employees, the management and the Animal Welfare Body are capable of working efficiently in undertaking the main assignment – ensuring optimal animal welfare. This require focusing on shared goals, a common language, knowledge-sharing, mutual respect and that communication is efficient, frequent, timely, accurate and has a problem solving approach. Trust – means that actions are consequent, transparent and explicable. Act according to what is said and inform on actions. Delegate responsibility and power. Listen to others' views and take it seriously. Integrity – means 'walk the talk'. Keep a consistent approach to raised issues. It should be possible to 'appeal' decisions. Decisions are made on the basis of all relevant available information. All involved parties should participate. The process must be in accordance with fundamental ethical principles. This value may have other names: justice, fairness, honesty, rightness.

OF11M4 Small Refinements = Large Enhancements to Science and Animal Welfare Optimizing the conduct and implementation of animal studies

Rensing, Susanne, Presenting author
Abbvie Deutschland GmbH & Co KG

Science continues to evolve at a rapid pace. Our understanding of laboratory animal behaviour and welfare is also growing.
The biomedical research community continues to receive criticisms from activists and concerned citizens that our use of the animals is not justified and our care of the animals is inadequate. These concerns are often not based on facts; however, we must continue to seek ways to optimize the conduct of animal studies by adopting the highest ethical standards and practices. Refinements to the way we care for the laboratory animals as well as the way we conduct our studies can lead to large enhancements to both science and animal welfare. Participating in public outreach to explain why we work with animal models and how we provide ethical care for them can help a misinformed public to understand the work we do. Those attending this webinar will learn about how to develop a culture of welfare that is continuously evolving through the implementation of refinements to housing, environment, enrichment, supportive care, humane endpoints, and training and innovative 3Rs initiatives. They will also be encouraged to take a more active role in public outreach to advance public understanding of biomedical research with animal models.

OF11M5 Survey by questionnaires developed from focus interviews in Danish laboratory animal facilities revealing striking differences between public and private sector scientists in their knowledge about the 3Rs

Lassen, Jesper[1], Presenting author.
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Nøhr, Rikke[1], Co-Author, Lund, Thomas B.[1], Co-Author, Knudsen, Lisbeth E., Co-Author, Ottesen, Jan L[2], Co-Author
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[2]Laboratory Animal Science, Novo Nordisk A/S, Denmark

Although the 3Rs were introduced more than 50 years ago, there is limited knowledge about their implementation and use in the research labs. Since the 3Rs has become a core principle in the recently revised EU directive on animal experiments (Directive 2010/63/EU), this knowledge is still more pertinent. This paper reports results from a sociological study financed by the Danish 3R Center about Danish animal scientists' knowledge, perceptions and experiences with the 3Rs.
Materials and methods based on qualitative interviews a questionnaire was developed and distributed online to scientists working with animal experiments in Denmark in Oct-Nov 2015. The survey included sections on knowledge, awareness, perceptions and experience regarding the 3Rs as well as knowledge of the Danish 3R Center. Statistical analysis of the data was carried out using the software SPSS.ResultsRespondents (N=234) represent a diversity in size and type across public and private institutions. 67% of the private sector scientists reported that they know the 3Rs very well; compared to 35% of public sector scientists. Results from a knowledge quiz showed good factual understanding of replacement and reduction with minor differences between the sectors. The understanding of refinement was poorer – with public scientist demonstrating a noteworthy poorer understanding. 23% of the public sector scientists considered 3Rs as part of their daily activities; compared to 54% of the private sector scientists. There was clear difference between the sectors in the overall view of the 3Rs – thus 53% from the private sector strongly disagreed that implementing the 3Rs would be detrimental to the quality of their research; compared to only 22% of the public scientists. The

majority of the scientists reported that reduction and refinement frequently or very frequently play a role when designing/ doing experiments (77% and 84%); while the same was true for 43% for replacement. 29% reported to have been involved in developing techniques to achieve replacement, while 69% had done so to achieve reduction and 78% to achieve refinement. Willingness to share data (71%) and change methods (41%) were seen as the most important obstacles to achieve reduction. Discussion and conclusions. Despite the overall good understanding of the 3Rs the striking differences between public and private sector scientists point to a need of strengthening communication and education about the 3Rs in particularly targeting the public scientists. Institutions like the Danish 3R Center may play an important role for this. Moreover, the study point to the need for facilitating scientists' ability to develop and implement means to achieve replacement, as this principle stand out as meeting most obstacles. To explore possibilities of replacement a similar survey could address perceptions and practices of researchers mainly working with non-animal models.

OSFE1 AALAS – FELASA: Partnering Across the Big Pond

A. Turner, Presenting speaker
American Association for Laboratory Animal Science

In 2012, the American Association for Laboratory Animal Science and the Federation of European Laboratory animal Science Associations entered into an agreement to jointly sponsor and participate in working groups. The group topics would be identified by the respective organizations Executive Committees and appointments to the group would be made by the respective presidents. The purpose of these groups is to deliberate salient topics and issues in laboratory animal science and make recommendations to benefit the community at large. The AALAS-FELASA liaison body has been productive thus far towards harmonizing practices and standards internationally. It appears to be a relationship that is withstanding the test of time and the normal turnover of leadership in both groups. Specific information about the working groups this far, including those that have completed their assignment, the ones that are in the "work in progress stage," and those that are beginning investigations will be presented.

Building on the success of the past four years, efforts have been initiated this year to continue to solidify this relationship. Topics of discussion have included:

- A more definitive publication path.
- A plan for future working groups with the intent of a defined number of active working groups at any one time.
- Increasing the number of teleconferences to discuss progress and future needs.
- Scheduling of face to face meetings at the national AALAS meeting or FELASA Congress as able.
- Formalizing the AALAS coordinating process to oversee this effort.

OS1WE Integrating Environmental Sampling into Animal Health Monitoring Programs (Sponsored by Idexx Bioresearch)

Riley, Lela, Livingston, Robert, Presenting authors
IDEXX BioResearch, Columbia, Missouri, US
Crim, Marcus, Co-Author

In rodent health monitoring programs, strategies to incorporate environmental monitoring are under evaluation for effectiveness in disease detection, ease of implementation, and cost. In this seminar, the advantages and limitations of various environmental and animal-based sampling approaches will be discussed. Examples of how to apply these testing approaches and interpret and confirm results are provided to facilitate adaptation of these methods to institutional health monitoring programs.
New strategies in the design and implementation of animal health monitoring programs complement the evolution of animal husbandry practices and trends in pathogen prevalence in research colonies. In rodent health monitoring programs, strategies to incorporate environmental monitoring are under evaluation for effectiveness in disease detection, ease of implementation, and cost. Environmental sampling can be performed at the cage and rack level and provides information that is complementary to the traditional monitoring practices of sampling colony and sentinel animals. In this seminar, the advantages and limitations of various environmental and animal-based sampling approaches will be discussed. Examples of how to apply these testing approaches and interpret and confirm results are provided to facilitate adaptation of these methods to institutional health monitoring programs.

OS3WE1 Advanced Cryopreservation Methods: New Ways to Manage Genetically Modified Mouse Colonies (Sponsored by Janvier Labs)

Taft, Robert, Presenting author
Director of Reproductive Technologies and Business Development, The Jackson Laboratory, USA

The growth in the number of genetically modified mouse strains in research is rising exponentially, putting pressure on institutions to find vivarium space, funds and staff to care for these important research models. Frequently researchers maintain strains that are not under active study resulting in breeding of animals simply to perpetuate the unique strain or to distribute it as required by their grant.
Exchange of mice models between researchers and institutions is burdensome. This talk will summarize how new cryopreservation methods have been used by The Jackson Laboratory to effectively manage and distribute genetically modified mouse models and to safeguard them from loss. Methods: The Jackson Laboratory has more than 20,000 unique strains of mice cryopreserved as either embryos or sperm. Of the 8,700+ strains we distribute, nearly 6,600 are maintained exclusively in a cryopreserved state and must be quickly and reliably recovered upon request. In addition, our collection of genetically modified strains we distribute is growing at a rate of more than 500 strains per year. We will present an overview of cryopreservation methods used to manage these colonies and the advantages and limitations of each method. Results: Data on how The Jackson Laboratory utilizes cryopreservation methods to accomplish the following objectives will be presented: 1) Reduce costs of managing a large portfolio of genetically modified strains; 2) Reduce genetic drift in inbred colonies via our Genetic Stability Program; 3) Reduce genetic drift in genetically modified strains; 4) Rapidly recover and cost-effectively distribute specific opportunistic and pathogen free (SOPF) mice; 5) Reduce the global expenditure of resources to create, characterize and breed research animals; 6) Reduce the number of animals used in biomedical research; 7) Safeguard strains from catastrophic loss. Conclusion: Development, characterization and husbandry of unique genetically modified mouse models of disease represent a large investment by researchers. Caring for these animals represents an ongoing cost of operations requiring increasing space, resources and resulting in rising quantities of animals be used in biomedical research. Recent advances in cryopreservation and recovery of sperm developed by The Jackson Laboratory permit investigators to implement new strategies that can greatly reduce the cost of maintaining these strains while preventing genetic drift, safeguarding them against catastrophic loss and enabling them to be quickly and cost effectively revitalized in large, age-matched lots of animals at a specific opportunistic and pathogen free health status.

OS3WE2 Reproductive sciences: another method to manage your breeding of transgenic rodents (Sponsored by Janvier Labs)

Guinut, Frederic, Presenting author
Reproductive Sciences Manager, JANVIER LABS, France

Transgenic rodents are now essential tools for research. The multiplication of models in laboratories leads to logistical, material and technical constraints, which can be an obstacle to scientific research progress. In order to overcome these constraints, there are solutions which combine reproductive sciences and customized breeding approaches, and will provide you with animals of high sanitary and genetic quality. This method will also be a powerful tool for cost-control, time management and rationalize the use of animals to completely integrate the principle of the 3Rs (Reduction, Refinement and Replacement). These services allow researchers to focus on their experiments, without losing control of the breeding management.

OS4WE Criticality of Genetic Quality in Genetically Modified Animal Models (Sponsored by Taconic Biosciences)

Perez, Ana V[1], Simon, Michele[2], Presenting authors
[1]Taconic Biosciences
[2]MRC Harwell

In this workshop we will present a couple of talks that will address the importance of genetic quality and how genetic background influences the phenotype of genetically modified animal models.
The abundance of genetically modified animal models is staggering and those numbers only continue to grow. The CRISPR technology has further shortened the time to generate genetically modified animal models. This on the one hand is good news for many researchers that are always on the look of the ideal mouse or rat model to model hypothesis but on the other hand many times these genetically modified models are on a mixed genetic background or different substrains, which many times complicates data interpretation. In this seminar we will present two talks that will provide information on different aspects of genetic quality when breeding genetically modified models. The first Presenting author will address what encompasses genetic quality of a genetically modified animal model and what considerations need to be addressed when intercrossing them. The second Presenting author will emphasize important differences that substrains carry and how the choosing of the substrain to use is not a trivial issue for the phenotype of the genetically modified mouse or rat models. Participants will learn that verification and maintenance of

genetic quality on their breeding colonies of genetically modified models is of utmost importance. They will become aware of tools that will help them with the verification of the genetic background of the rat or mouse model being used. They will also learn on potential pitfalls of experimental design of genetically modified models and that phenotype may vary when using different sub-strains.

OS5WE Health monitoring sample collection - 3R innovative, non-terminal techniques

Udobi, Adaobi, Foa, Massimo, Presenting authors
IDEXX BioResearch Europe

Learn how health monitoring can be conducted on non-terminal samples from rodents.
- Including hand-on training on Dry Blood Spot (DBS) and swabs.
- Gain insight in testing methodologies like MFI2, RT-PCR and MALDI-TOF
- Q&A session

OS6WE Electromagnetic field (EMF) technology has the potential to improve both scientific data capture and welfare assessment in animal facilities by allowing automated data collection and digitalization (e.g. animal activity) from individual cages minimizing impact on animal welfare

McCormick D, Burman O, Recordati C, Prins JB, Kiliaan A, Presenting authors

This seminar is provided for vivarium managers, facility veterinarians and technical staff to help them appreciate how new Electromagnetic Field, and Extremely low Electro Magnetic Field (EMF/ELF) technology and associated software, integrated into standard cage racks, can, by continuous, automated home cage environment monitoring and data capture, support animal care and scientific programs, while reducing animal stress and operating costs. As vivarium operations increase in size and complexity, EMF technology will become more pervasive and cost effective. Recent developments and applications of EMF/ELF evaluation in regards to the animal welfare and scientific data capture will be discussed.
The Seminar will show from first experiences, how electromagnetic Field (EMF) technology has the potential to improve both scientific data capture and welfare assessment in animal facilities by allowing continuous automated home cage data collection (e.g. animal activity and aspects of the cage micro-environment). The Seminar will prove, describing Histopathology and Behavioural data in relationship to continuous EMF exposure mice studies, that the EMF technology does not have any potential influence on either on animal welfare or research outcomes. Previous unrelated EMF studies found contrasting results of different durations and/or intensity on laboratory rodents. These differences in exposure meant that independent preliminary clinical pathology and behavioural studies were required during the development of a new continuous low EMF automated home cage monitoring system. The methodology and results of these investigations are reported in this seminar.
These technologies improve communications and staff efficiencies thereby increasing both the quality and quantity of animal care and ultimately positively modify outcomes of animal welfare. Current applications of EMF technology specifically developed for continuous 24/7 monitoring of the home cage microenvironment and laboratory sources of EMF together with potential effects on humans and animals will be assessed. Delegates will be updated on new and innovative developments and methods of micro environmental monitoring and management of rodents. Data from the results of trails of long term continuous exposure of mice to low EMF in relation to normal behaviours, clinical pathology, growth rates and breeding performance will be provided to support end-user acceptance and system validation.
Combined low EMF technology and innovative software algorithms support improved animal welfare, greater operational efficiencies, and reduce the environmental footprint of animal care operations. Examples of this technology used in the management of transgenic rodent models, with greater varieties of animals with much more complex care and welfare concerns. Costs of maintaining these animals and providing appropriate care are major institutional concerns.
Developments in EMF technology aim to provide non-invasive, automated home cage monitoring systems to provide 24/7 animal care programs in support of science and animal welfare. Each of these areas will be reviewed in depth to allow delegates to assess the opportunities provided by this revolutionary technology.

OS7WE The Vital Role In Vivo Research Plays in Drug Discovery – Personal Experiences From Today's Translational Science (Sponsored by Charles River)

Cortese, Riccardo[1] and Guidotti, Luca[2], Presenting authors
[1]Okairos, Rome, Italy
[2]Immunopathology Unit, Ospedale San Raffaele (OSR), Milan, Italy

This workshop will present an inside look at how in vivo science plays a crucial role in modern drug discoveries. Our two Presenting authors will present an informal and personalised account of their research, and how in vivo models and translational medicine have contributed in their successful efforts towards finding effective vaccines and therapies for human and animal diseases.
Dr Riccardo Cortese has coordinated the efforts leading to the development of several novel antiviral drugs and vaccines including those which battle respiratory syncytial virus, Hepatitis C, Malaria, tuberculosis, Ebola and HIV; many of which are already on the market (Raltegravir - ISENTRESS) or at various stages of clinical development. He firmly believes that during the next decade, a new generation of T-cell based vaccines will become the major medical contribution in the fight against infectious disease.
Dr Guidotti will discuss the role of in vivo research for the hepatitis B virus (HBV). His efforts with different HBV transgenic lineages (including HBV-replication competent transgenic mice) has generated many interesting and encouraging outcomes, including the possibility to test safety/efficacy of antiviral drugs in relevant and manipulable animal models of HBV infection. The very few antivirals (e.g. nucleoside analogues) that have received FDA approval in the last 15 years have all gone through various stages of development in animals models.
After the talks from our guest Presenting authors we will welcome the opportunity to answer questions from our audience. All attendees will be able to receive Charles River's complimentary poster on Common Health Conditions, which has been translated into English, French, Spanish, German, Italian and Portuguese.

OS8WE A review of the evolution of animal health surveillance from the bedding sentinel method to exhaust air dust (EAD) PCR (Sponsored by Charles River)

Henderson, Kenneth[1], Durand, Stephanie[1], Kerton, Angela[2], Presenting authors
[1]Charles River
[2]Imperial College

Though publications have long supported the fact that many pathogens do not readily transfer to bedding, the soiled bedding sentinel method remains the standard practice for animal health monitoring.
Despite the supporting data, this method has gone unchallenged in research vivaria for more than half a century; it is only in recent years that alternatives have been developed and integrated into health monitoring programs. One such method that has quickly gained acceptance is PCR-based quarantine, which is now being adopted for the routine screening of rodent colonies. This workshop will highlight the recent advances in health monitoring that promote the 3Rs while improving pathogen detection. Topics include the use of the dry blood spot and polymerase chain reaction (PCR) testing to replace or supplement the bedding sentinel method, and the introduction of exhaust air dust (EAD™) PCR. The workshop will conclude with a case study detailing a research animal facility's transition from the use of sentinels to PCR-based health monitoring.

OS9WE Sterilizers for BioContainment Applications - Challenges and possibilities for sterilizers in BioContainment applications.

Joakim Larsson, Speaker
Product Line Manager Life Science Sterilizers

Like all laboratories and research facilities, bio-containment laboratories need autoclaves for sterilization and decontamination. The pathogenic nature of the waste material from such facilities coupled with the use of the autoclave as a barrier between the facility and the outside world places special requirements on the autoclave design and processes used that standard autoclaves cannot fulfill.
Barrier System
In biocontainment applications sterilizers are used as a pass-through between zones and in this application they are also an integral part of the building containment system. Typically a
double door chamber forms a barrier between zones of different classifications. As such, the chamber must be sealed to the wall to form a barrier. The different solutions (depending on type of biocontainment) application will be discussed, these range from traditional air differential barriers to a true bioseal that guarantees an air tight seal even in BSL3/4 environment with the highest containment requirements.Pressure transducers and gauges are diaphragm isolated sanitary design to be protected against stagnant water in the system.
Door sealing mechanism
The doors can be sealed either using a traditional active door gasket or a system that mechanically locks the door. The active gasket is mechanically simple and inherently reliable while the mechanical system is somewhat more complex but does not rely on utilities as compressed air, steam or electricity to maintain the seal between chamber and door, it can keep the chamber integrity for an extended time.
Process System
The balance between sterilization decontamination efficacy versus containment presents several challenges to the sterilization system designer. The demand of air removal prior to steam injection is established as a principle of steam sterilization. This is a challenge since air in contact with materials in the chamber is contaminated and cannot be removed without treatment.
Another challenge is the physical principle that steam condenses when it comes in contact with cooler surfaces. The condensate produced as steam heats the contaminated materials, prior to achieving sterilization decontamination conditions, is itself contaminated. It requires treatment prior to release to the building drain.

Several options are available to treat the removed air and condensate, and should be applied based on a risk assessment of the facility design:
Effluent Retention Filter Option
Air evacuated from the chamber can be filtered using a 0.22µm sterile membrane filter. This renders the air sterile and the filter is steam sterilized during the subsequent process. For added security, a second filter may be installed in the series.
Additionally, an automatic in-place Water Intrusion Testing (WIT) integrity test may be performed on the filtration system. Condensate produced is collected in the chamber base and is heated by incoming steam and by the steam heated external jacket.
Sterilization decontamination of the condensate is confirmed by temperature sensors.
Incinerator Deactivation Option
Pass the air through the unique Getinge incineration system. This validated high temperature device provides a torturous pathway for the evacuated air. This destroys all viable organisms, rendering the exhaust air sterile and safe to discharge. Condensate is treated as described for the filter option.
Fumigation of Process System
How to prepare the sterilizer for maintenance, an inoperative sterilizer, filter or incinerator that requires service or repair must be free from any contaminants in order to protect service personnel and non-controlled areas. There are ports, valves and procedures to enable system fumigation, e.g. with steam, formaldehyde gas or hydrogen peroxide. The decontamination of the process system allows for safe maintenance or emergency service of the sterilizer chamber and filter/incinerator

Summary of content
1. Interfaces to the building
a. Barrier function
i. Cross Contamination Barrier
ii. Bioseal
b. Utility connections incl. drain
2. Sterilizer Mechanical design
a. Sterilizer door and sealing system
b. Chamber and barrier penetrations
3. Process Considerations
a. Steam Sterilization
b. Effluent deactivation
i. Filter
ii. Incinerator
c. Condensate handling
d. Emergency decontamination
e. Using the Sterilizer Chamber as H2O2 pass-through
4. Workshop
a. Practical exercise in sterilizer selection for both lab and animal research facilities with different BSL classifications.
5. Good questions to ask yourself and your supplier before buying a sterilizer for a BioContainment application
For the workshop both printed material and interactive app supported assistance in an iPad will be used.

OSFE11 FELASA today and tomorrow - a growing pan-European scientific network

Brandstetter, Heinz, Presenting author
FELASA, President

The Federation of Laboratory Animal Science Associations unites the European national and regional laboratory animal science associations. It is managed solely by representatives of its constituent associations and combines and coordinates the expertise of its member associations. Over the last decades, FELASA has evolved into a truly pan-European scientific organization with 21 Laboratory Animal Science (LAS) Associations as members representing 27 countries. Rus-LASA, PolLASA, and SLAS were the three last associations joining FELASA in 2014 and 2015 respectively. FELASA is representing the views and opinions of the European LAS community as a stakeholder with the European Commission and the Council of Europe in Brussels and maintains relations with national and international bodies and associations concerned with laboratory animal science. All activities of FELASA are in accordance with the "Three 'R's of Russell & Burch (1959)" (Replacement, Reduction, and Refinement). FELASA advocates responsible scientific conduct with animals in the life sciences. FELASA publishes recommendations and best practice guidelines in order to improve the welfare of the animals used for the advancement of science, human and animal health, as well as public and environmental safety. In this context, FELASA recognizes the ongoing need to use animals for the benefit of patients.
Another main activity of FELASA is to drive towards professional competence in all personnel working with animals and facilitating the mobility of researchers within the EU. FELASA developed already in the 1990s a scheme for education and training based on the European Convention ETS 123, Article 26 (Council of Europe) and the Council Directive 86/609/EEC, Article 14 (European Union). FELASA's Accreditation board for education and training revised this scheme according to the requirements stated in article 23 of the EU Directive 2010/63 and the EC Guidelines (European Commission, 2014). FELASA accredits courses (not people) that deliver training for all categories of personnel involved in the care and use of laboratory animals, i.e. Functions A, B, C & D defined in article 23 of EU Directive 2010/63. Accreditation will also be available for the education and training of Designated Veterinarians, Project Evaluators and what we define as Specialists in laboratory animal science (Specialist in LAS) - a person who may be involved in tasks described in articles 24 and 25 of EU Directive.
FELASA relies almost entirely on voluntary work by experts appointed by its constituent associations. As for FELASA's work the number of volunteers can hardly be large enough, additional expertise, views, input and support are always more than welcome. Therefore, do not hesitate to contact us and offer your active participation through your national Laboratory Animal Science Associations: www.felasa.eu, email: info@felasa.eu.

OSFE12 Impact of FELASA working groups on animal welfare and experimental research

Eklof, Ann-Christine, Presenting author
FELASA, Vice-President Working Groups

FELASA is a federation for European laboratory animal associations. This organizations has been working since it was established in 1978
FELASA puts the 3Rs of Laboratory Animal Science 'Replacement, Reduction and Refinement' in center. FELASA also advocates responsible scientific conduct with animals in the life sciences with particular emphasis on ensuring animal welfare.
The establishment of FELASA working groups is one of the most important tasks for FELASA. These working groups give the possibility to harmonize among the member countries.
Many of the working groups have been crucial for the animal welfare and for secure good standards to perform good and reliable science. FELASA has produced guidelines and recommendations for more than 15 years.
The Working Groups consists of specialists in each of the addressed topics, and are nominated by the FELASA constituent associations, and after going through CV and recommendations elected by the FELASA Board of Management.
A good example of what a WG has produced is the guideline for health monitoring of rodents, which is very important for the exchange between different institutions and countries. In many research institutes and animal breeders it is compulsory to follow this guidelines when importing and exporting animals.
FELASA and AALAS established some years ago a liaison. 3 working groups were established and two of them have already published recommendations and the third about Harm Benefit analyses is ready and will soon be published. There are new working groups working within this liaison. These guidelines and recommendations have influenced the development of various regulatory requirements in Europe, including those related to education and training, routine laboratory animal activities, and animal health monitoring.

OSFE13 International Liaisons: FELASA projection beyond European boundaries

Pintado, Belen, Presenting author
FELASA, Vice-President International Liaisons

FELASA, the Federation of Laboratory Animal Science Associations, represents common interests in the furtherance of all aspects of laboratory animal science (LAS) in Europe and beyond. As a federation, one of the goals is to harmonize a common understanding among its members of responsible scientific conduct with animals in the life sciences with particular emphasis on ensuring animal welfare and implementation of the 3Rs. FELASA is the common voice of 21 member associations representing 28 European countries. This provides a strong representative role in the relations with national and governmental bodies in Europe. As a result, it has been recognized as main stakeholder by the European Commission and the Council of Europe in matters related with laboratory animal science in Europe
In a globalized world, FELASA's role cannot be limited by the European borders and an international projection has been sought for a number of years, expanding contacts and collaborations with international organizations with coincident goals like ICLAS, with national associations outside the European space, like AALAS or global accreditation organizations like AAALAC. More than a mere formal recognition, FELASA seeks collaboration in specific topics that will help to promote our better understanding and implementation of common goals towards a better laboratory animal science and improved animal care.

OSFE15 ICLAS-FELASA collaborations and achievements

Vergara, Patri, Presenting author
International Council for Laboratory Animal Science, President

The International Council for Laboratory Animal Science (ICLAS) is a global organization to promote high quality science by means of promoting high quality defined animal models and by promoting education and training of personnel involved in the work with animals. ICLAS is very well known for its harmonization role and for the promotion of education and training, particularly in developing countries. Furthermore, ICLAS plays also a role providing tools to improve the quality of the animals used in research by means of the Network for the promotion of laboratory animal quality (LAQN).
FELASA and ICLAS share aims and objectives and for this reason both organizations stablished a liaison body in 2014. Two areas have been established as a priority: 1) to promote courses in those areas of Europe where there is a need for expanding education and training; 2) for ICLAS to contribute to FELASA working groups promoting genetic quality of laboratory animals. In addition, ICLAS will continue with the European Fellowship training program, a hands-on training opportunity addressed to individuals from European countries/areas in the process of developing modern standards of animal care and use.

PA1 Are Sham Operations Necessary?

Atkinson, John, Presenting author
UCB

The 5/6th Subtotal Nephrectomy model is a widely used, hypertension-driven model of CKD. It is characterised by initial tubulointerstitial fibrosis and glomerulosclerosis, leading to End Stage Renal Failure in 60-90 days. Normally, the control animals in these studies have a 'Sham' operation. We sought to determine if this surgery was necessary, with functional and histological comparisons between Sham animals and Non-Operated animals
Chronic Kidney Disease (CKD) currently consumes around 5% of the NHS budget in the UK and is set to rise with increasing numbers of patients requiring renal replacement therapy. In addition to the financial burdens imposed, CKD can be severely debilitating, greatly reducing the quality of life for the affected patient. Most forms of CKD progress to End Stage Kidney Failure (ESKF) through progressive fibrosis of the organ. This fibrosis is characterised by an expansion of both the glomerular and tubular basement membranes, leading to glomerulosclerosis and tubulointerstitial fibrosis respectively. There is also increasing clinical evidence to identify CKD as an independent risk factor for coronary heart disease and cardiovascular events. The two most commonly used models of renal fibrosis are the subtotal nephrectomy (SNx) model, and the unilateral ureteral obstruction (UUO) model. The UUO model is a relatively simple model of renal fibrosis which is performed by tying off the ureter of the left kidney allowing urine backflow and resultant fibrosis similar to obstructive nephropathy. This model allows no functional data to be collected as the right kidney remains functional in the animal. The 5/6th Subtotal Nephrectomy model is a widely used, hypertension-driven model of CKD. It is characterised by initial tubulointerstitial fibrosis and glomerulosclerosis, leading to End Stage Renal Failure in 90-120 days. Other models, such as the Unilateral Ureteral Obstruction (UUO) or the Folic Acid (FA) model are excellent tools for initial screening, but neither mimics the progression of CKD in patients as well as SNx. Normally, the control animals in these models have a 'Sham' operation. We sought to determine if this surgery was necessary, with functional and histological comparisons between Sham animals and Non-Operated animals, and comparisons between these and the contralateral right kidney which remains in the UUO model.For the SNx model, we found no significant difference between a sham-operated animal and a non-operated animal in terms of function (serum creatinine, creatinine clearance, albuminuria, blood pressure) or histology (Masson's Trichrome or Sirius Red staining). In the UUO model we found no difference in histology between a sham-operated animal and a non-operated animal. We feel, therefore, that in the interests of animal welfare and the 3Rs, that exposing animals to the stress of sham surgery is unnecessary.

PA2 Optimization of permanent venous catheter sampling in LYD pigs

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The LYD pig is often used for pharmacokinetic investigations after s. C. or i.v. injections of test substances. In some studies, there have been signs of cross talk wich gives less precise data. We have optimized our venous catheter sampling method, to decrease the variance and thereby decrease the number of animals used.

Materials and Methods: In order to sample blood LYD pigs are routinely catherised with permanent venous ear catheters. This is a widely used method which is easy and safe to perform. In some types of studies, where rapid infusions have been performed in a parallel vein or where ultra-rapidly absorbed insulins have been administered subcutaneously, there have been signs of cross talk. When this happens, blood from the opposite Vena Jugularis, where the infusion of test substance is performed, enters the catheter and thereby gives less precise data. This effect is to some extent known but it is not always taken into consideration in the design of pharmacological and toxicological studies. The problem has been, that a catheter length of 42 cm has been used, regardless of the size of the pig. Autopsy has shown that this 42 cm catheter is not always isolated to the Vena Jugularis, but often enters the Vena Cava. We therefore tested shorter lengths of catheters spanning from 32 to 26 cm in length. When the catheter decreases in length the veins get smaller which can make blood sampling difficult. On the other hand the catheter should be short enough to avoid cross talk. We conducted four studies with LYD pigs receiving a s. C. injection in the neck area of a formulation with faster-acting insulin aspart 0.6 nmol/kg with blood sampling through an ear vein catheter placed in Vena Jugularis on the opposite side. Animals were between 65 kg and 100 kg and fasted for 18 hours. The pigs were catherised in an ear vein for blood sampling. The catheterisation is performed using the Seldinger Wire Technique and the catheters were either 27 cm or 42 cm. Results: We found from the pharmacokinetic profiles of the two groups, that the shorter catheters in 27 cm, removes cross talk from the infusion, when the opposite vena jugularis is used. Furthermore, we found a decrease in variance for the most common primary endpoints in studies where s. C. administration were used. Depending on the study, this has decreased the number of animal needed when conduction power calculations and we can save 20%-40% animals in our studies. Discussion:This method has now become standard for all relevant studies in Novo Nordisk. We expect this will reduce the number of animals used. Conclusion: Venous catheter position is crucial for blood sampling after s. C. injection of compounds. We have shown how optimising the sampling procedure can obtain better data and thereby reduce the number of animals used.

PA3 Vascular Access Port placement when implanting a catheter in the saphenous vein of Göttingen Minipigs - Challenges in transferring a protocol from one species to another

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Ellegaard Göttingen Minipigs
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Vetsalius

As serial infusion and blood sampling are often important technical aspects of an experimental design, vascular access is a prerequisite. Göttingen Minipigs have a convenient size for handling, but restraint and venipuncture can be stressful and affect blood parameters. When experiments require frequent blood sampling, catheterization is the best option, both ethically and scientifically. VAPs are ideal for long term access; this study aims at reducing invasiveness of the implantation procedure.
A Catheter with VAP is a fully implanted system and is designed for long-term vascular access. The advantage is that animals can be group housed, as there are no bandage and no exterior parts. A VAP is helpful to collect serial blood samples from Minipigs and for repeated i.v. dosing. Implanting Catheter with VAP is common in various species including human. Sites used in Minipigs are:- Insertion in external V. jugularis with port around shoulder or back- Insertion in V. femoralis with port on the hip. In studies with NHP, catheters have been implanted in V.saphena,

with the tip of the catheter in V. Cava caudalis and a port placed around the knee. This was found to be less invasive, faster to implant, and led to better recovery - a refinement in the sense of the 3R's. The aim of the present study was to transfer this technique from NHP to Minipigs and was designed as proof of concept. Initially 5 male Göttingen Minipigs, aged 10 months were included in the study. After a single incision, the vein was isolated, the catheter inserted, tested and the attached port placed in a subcutaneous pocket laterally to the incision site and then closed up. The procedure was quick and smooth and recovery was uncomplicated. However, the location of the port craniolateral from the incision site, on top of the tibia proved suboptimal as all pigs developed skin abrasions when they were resting or sleeping. The abrasions were at the site where the septum of the port was located and affected access to the port. This was assessed to be a humane endpoint, and subsequently other sites for the port were tested. We realized that applying a protocol from one species to another is not always straight forward. There were no issues at time of implantation. Selecting port sites however is very important. NHP are athletic and can reach almost all sites with their hands; one criteria when choosing a site. A Minipig is not able to interfere with their hind legs so the chosen site was judged safe. Ease of access to a VAP is assisted by placing the ports on top of a hard anatomical structure to provide resistance for penetration. In this case, it was counterproductive as the lesions were caused by the interaction of the bulge that the port created and the floors in the pen. The extend depends on the character of the floor, but it is amplified by the fact that the port provides resistance to pressure. When choosing alternative port sites we needed to balance ease of access and risk of injury.

PA4 Novel rat models to study primary genital herpes simplex virus-2 infection

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Envigo.

In this study we describe six rat models (SD, WIST, LEW, BN, F344 and DA) that are susceptible to intra-vaginal herpes simplex virus-2 (HSV-2) infection, after pre-treatment with progesterone.
AbstractIn this study we describe six rat models (SD, WIST, LEW, BN, F344 and DA) that are susceptible to intra-vaginal herpes simplex virus-2 (HSV-2) infection, after pre-treatment with progesterone. At a virus dose of 5 × 106 PFU of HSV-2, all rat models were infected, presenting anti-HSV-2 antibodies, infectious virus in vaginal washes, and HSV-2 DNA genome copies on lumbosacral dorsal root ganglia and the spinal cord. Most of the LEW, BN, F344, and DA rats succumbed to systemic progressive symptoms at day 8-14 post infection, but presented no or mild genital inflammation while SD and WIST rats were infected asymptomatically. Infected SD rats did not reactivate HSV-2 spontaneously or after cortisone treatment. We also investigated whether an attenuated HSV-1 strain (KOS321) given intra-vaginally, could protect from a subsequent HSV-2 infection. All LEW, BN, and F344 rats survived a primary HSV-1 infection and no neuronal infection was established. In BN and F344 rats, anti-HSV-1 antibodies were readily detected while LEW rats were sera-negative. In contrast to naïve LEW, BN, and F344 rats where only 3 of 18 animals survived 5 × 106 PFU of HSV-2, 23 of 25 previously HSV-1 infected rats survived a challenge with HSV-2. The described models provide a new approach to investigate the protective effects of anti-viral microbicides and vaccine candidates, as well as to study asymptomatic primary genital HSV-2 infection.

PA5 Intestinal inflammation after 6 minutes of cardiac arrest in rats - cytokine profiles in jejunum and serum

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Cardiac arrest (CA) induces a systemic inflammatory process called post-cardiac arrest syndrome (PCAS) (1). While the effects of PCAS in brain are well-known the role of the small intestine in systemic inflammation after CA is not completely understood. Therefore, the aim was to evaluate inflammatory processes after CA in intestinal tissue and sera by cytokine profiling in an in vivo rat model.
METHODS: Male Wistar rats, aging 7-8 weeks and weighing 280-320g, were subjected to 6 minutes of CA followed by cardiopulmonary resuscitation (n=4-6) and compared to a sham group (n=4). Intra-cardial blood was collected and a 2-cm-long segment of the proximal jejunum was excised at 6, 24, 72 hours and 7 days post CA. A multiplex cytokine assay (Bio-Plex, Bio-Rad, Hercules, CA, USA) was performed using the Bio-Plex Pro Rat Cytokine Th1/Th2 panel to measure cytokines in the jejunum lysates and serum. The data were analysed by one-way ANOVA and the Tukey's multiple comparison test. The level of significance was set at P=0.05. RESULTS: In jejunal tissues, levels of IL-1α, IL-1β, IL-10, and TNF-α showed significant peaks at 24h and were 1.5 to 5.7 times higher than levels at 6h post CA and in the controls ($P < 0.05$). At 72h, these cytokine levels were 1.5 to 2.5 times lower than the levels observed at 24h. Although no significant differences were found in the level of IL-6 in serum its level decreased significantly in jejunum lysates at 72h (0.27 pg/ml) compared to 24h (0.32 pg/ml). In serum, a significantly higher amount of only IL-1β was detected at 24h (15.78 pg/ml) compared to the controls (9.76 pg/ml).DISCUSSION AND CONCLUSIONS: Although there was a significant increase in IL-1α, IL-1β, IL-10, and TNF-α in the jejunum at 24h post CA cytokine serum levels were not significantly altered except for IL-1β after 24h. These results indicate that the observed intestinal inflammation may not be associated with systemic inflammation and hence, PCAS. Taken together, 6 minutes of CA resulted in changes in cytokine profiles primarily in the jejunum, indicating severe pathological consequences of CA on small intestinal tissue integrity.

PA6 IMPC - A Comprehensive Mammalian Genome Catalogue

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The International Mouse Phenotyping Consortium (IMPC) is building the first truly comprehensive functional catalogue of a mammalian genome by producing and characterizing a knockout mouse strain for every protein-coding gene.
Data from a standardized, broad-based phenotyping pipeline is collected and archived centrally by the IMPC-Data Coordinating Center. Dedicated 'data wranglers' are working with each phenotyping center to ensure proper transfer and quality control of data. A sophisticated statistical analysis pipeline identifies knockout strains with significant changes while accounting for bias from confounding effects. Annotation with biomedical ontologies allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research and facilitates integration

with other resources. The IMPC resource adheres to the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines, which lay out the reporting requirements to ensure all the information is available to allow reproducible research and minimise overlap in strain production. MRC Harwell has been a key player in the IMPC project and is aiming to complete generating and phenotyping 520 lines in the first phase. With phenotype data now available from all the centres for over 3000 genes at mousephenotype.org, we will focus on the new insights the IMPC is providing not only in identifying novel gene function but also identifying new functions for known genes.

PA7 Effect of a non-hypocholesterolemic atorvastatin treatment on inflammatory parameters during early stages of atherosclerosis: an animal study on WWHL rabbit

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It is commonly accepted that the atherosclerosis is an inflammatory process and that its evolution occurs hand-in-hand with inflammatory process at each stage of the disease [Ross, 1999]. Here, we propose to explore inflammatory circulating parameters evolution with age and treatment through the utilisation of an animal model, Watanabe rabbit.
In this study, we hypothesized that a non-hypocholesterolemic atorvastatin treatment at long-term has an effect on the inflammatory mechanisms implicated in atherosclerosis development. An atorvastatin treatment (2.5 mg/kg/day) was tested against controls on 34 male 3 to 12 month-old rabbits. This treatment had neither effect on LDL or LDLox circulating levels. The inflammatory state was assessed by the monthly measurements of CRP, IL10, IL-6, TNFα, INFγ, P-selectin, MCP-1, Tissue Factor, ICAM-1 and VCAM-1 blood circulating levels. Except for VCAM-1 and IL-6, no change in concentrations of these parameters was observed in control and treated group. A significant increase in VCAM-1 and IL-6 circulating levels was observed at the 6-9 month period in control group. Atorvastatin intake prevented this increase. The evolution of atherosclerosis was followed by OCT imaging and the 890 plaques observed were ranked in 4 classes depending on the severity of the lesions. Moreover, the surface occupied by macrophages was determined for each class of plaques. Our results show an evolution of atherosclerosis throughout the experiment in control group. This evolution is delayed by atorvastatin intake. This molecule could act both on macrophage recruitment and smooth muscle cell proliferation. In conclusion, this study highlights plasmatic inflammatory parameters modifications confronted with the atherosclerosis severity in a relevant model of cardiovascular diseases.

PA8 Infection promotion models of chronic osteomyelitis in rabbit - a comparison study

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Osteomyelitis is a serious problem caused by bacteria in the bone especially in children and elderly. Despite intense research efforts, in the past years no important progress has been registered in curing osteomyelitis. In this context, in vivo test models of the disease are greatly needed. However, reproducible osteomyelitis animal models are difficult to obtain as the animals either die shortly after inoculation of bacteria or the bone cures itself of infection.
Aim. The specific aim of the present study was to compare the potential of a series of foreign bodies used as infection promoters to induce chronic osteomyelitis without using sclerosing agents. Materials and Methods. Four groups of 10 rabbits each were infected with Staphylococcus aureus suspension and then inserted foreign bodies (steel wires, balls and cotton meshes) in their left tibia. Disease progression was monitored using various methods such as: direct clinical observation, haematological parameters, microbiology, histology and radiology. Results. The disease reproducibility rate was the lowest in the group where steel wires were used (60%) and the highest in the cotton meshes group (90%). Histopathological results evidenced osteolytic processes characteristic to either acute or chronic osteomyelitis. On the 50th day after inoculation, the cotton mesh group had chronic osteomyelitis, while the other groups still displayed acute osteomyelitis. Hematological results showed leukocytosis in the acute form in all groups and low neutrophils count and rate for the advanced chronic form. Dynamic radiological observation also supported occurrence of the chronic form with bone neoformation earlier in the cotton meshes group than in the other groups. Conclusions. The group of rabbits where cotton wool meshes were used as foreign bodies was observed to be the best model for development of chronic osteomyelitis.

PA9 Novel drug delivery approach: Refinement of an old technique

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This poster describes a novel method to delay compound delivery from an Alzet osmotic minipump by exteriorisation of a cannula attached to the pump. This cannula can then be removed when compound delivery is required. This is a more accurate method of compound delivery and utilizes a smaller more flexible tubing which is ultimately removed and does not remain implanted. This is beneficial to the welfare and comfort of animals and is a refinement to the current recommended "Lynch coil" method.
Materials & Methods Novel exteriorisation method: This method requires good aseptic surgical techniques· Alzet osmotic minipumps were filled with compound solution in a class II fume hood under sterile conditions.· The cap covering the flow moderator of the minipump was removed and sterile polyethylene tubing was connected (approx. 2cm used).· Under general anaesthesia minipumps were implanted subcutaneously into Balb/c mice (n=4 in total) with the cannula facing outwards through an incision on their back. · The cannula is placed to one side of the incision and the wound is closed with Vetbond.· Cannula should be exteriorised and held in place.· The cannula was then trimmed to a suitable length.·

Mice were given analgesia for 48h post surgery.· 72h post implantation mice were anaesthetised and the cannula was clamped and gently removed from the implanted minipump.· The wound is then sealed with Vetbond.· Blood samples were taken at 0.5h prior to and 3, 6, 24, 72, 120 and 168h post cannula removal to determine compound exposure.· Mice were monitored closely throughout studies and weighed/distress scored daily. ResultsNo compound was detected in mice 0.5h prior to cannula removal. At 3h post cannula removal compound was detected in the circulation from all four mice. This clearly shows that compound is only detected once the cannula has been removed. Steady state exposure was also maintained over the 168h study. In addition, body weight post implantation was maintained in mice undergoing this procedure.Discussion and conclusionCurrently the recommended technique to delay compound delivery from an osmotic minipump is to use the Lynch coil method. This method has limitations in terms of sterility, rigidity of tubing, size of minipump implanted, duration that tubing is implanted and the accuracy in the timing of compound delivery in vivo. The novel exteriorisation method outlined in this poster utilises a shorter more flexible tubing which is ultimately removed. In addition, this technique reduces the total size of the implanted device. The new method also has the potential to decrease the risk of infection and to deliver compound more accurately. This represents a potential refinement to the Lynch coil method and has clear welfare advantages for animals undergoing this type of procedure. It also has the potential to replace the Lynch coil method.

PA10 Hematologic characterization of blood samples from gene modified mice based on the B6 strain

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Phenotypical characterization of gene modified mice is used to identify physiological or pathological changes caused by mutations in mice. Hematological parameters are used for diagnostic evaluation, drug safety assessment and research studies, and may be used for health assessment before anesthesia and surgery. Knowledge about the normative values for a certain strain is thus essential for a correct interpretation of findings in gene modified mice.
Materials & methods: EDTA stabilized blood samples from 22 gene modified mouse strains based on B6N or B6J strains were taken in the period 2012-2015. The samples were taken from the periorbital venous plexus by EDTA coated pipettes and stored in K3-EDTA Microvettes while the animals were anesthetized with ketamine (100 mg/kg IP) and xylazine (10 mg/kg IP). The included animals were euthanized by cervical dislocation after the sample had been taken. Standard hematologic parameters for erythrocytes (RBC, HGB, HCT, MCV, MCH, MCHC), leukocytes (WBC, LYM, LYM%, MON, MON%, GRA, GRA%, EOS%) and thrombocytes (PLT, MPV) were analyzed on a Vet ABC Hematology Analyzer (Scil Vet, Germany). Samples from ten mice of each strain were included and mean value, standard deviation and lower and upper quartiles were calculated. A license was permitted by the Danish Animal Experiments Inspectorate. Results: The values for all parameters were within normal range (1, 2), although differences between the strains could be detected. Mice from the KIT W-sh, FceRI KO, Dmd and Dlk1 strain were found to be in the upper quartile for most parameters, whereas Fibcd1 KO, Dmbt1, mPAP4 and TH-GFP were found to be in the lower quartile for most parameters in this study. No consistency could be found in the ranking of the mouse strains and the parameter.Discussion and conclusion: All gene modified mice strains were within normal range for mice for all hematologic parameters. Although differences in hematologic parameters of gene modified mice could be found, no consistent ranking of strains and the parameters was present. The mice strains we included in this study had a normal hematologic profile, but strain differences were observed.

PA11 Refinement of the paracetamol method for measurements of gastric emptying in rodents

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Paracetamol is often used to measure gastric emptying in humans and rodents. This method is based on the principle that paracetamol absorption occurs completely in the small intestine, with negligible absorption in the stomach. In rodents, paracetamol can be measured using enzymatic assays, however; the large plasma volumes needed per measurement limits the number of blood samples per animal. From a scientific and ethical point of view smaller blood volumes would be beneficial.
Objectives: The aims of the study were to validate the method for measuring gastric emptying in rodents and to improve the assay for analyzing paracetamol in blood. Three experiments were performed: 1) Determination of the optimal p.o. dose of paracetamol in rats and mice; 2) Validation of gastric emptying model using atropine (muscarin receptor antagonist) and carbachol (muscarinic receptor agonist) in mice; and 3) Development of a LC-MS method for measurement of paracetamol in only 5 µl full-blood. The LC-MS method was compared to an assay run at a Cobas Analyzer (demanding 90 µl plasma). Methods: In the paracetamol dose-finding study (Experiment 1), overnight-fasted rats (Sprague Dawley) and mice (C57BL/6J) fasted for 5 hr were given an oral gavage (t=0 min) with paracetamol (25, 50, 75 or 100 mg/kg). In the validation study (Experiment 2), mice (fasted 5 hr) were injected with atropine, carbachol or vehicle before an oral dose of paracetamol (50 mg/kg). In the final experiment (Experiment 3), overnight-fasted rats were dosed p.o. with paracetamol after s. C. injection of vehicle or a glucagon-like peptide-1 analogue (GLP-1; inhibits gastric emptying). In all three experiments, blood was sampled from the tail vein (150 µl for rats and 5 µl for mice) at time 0, 15, 30, 45, 60, 90, 120 and 180 min. The concentration of paracetamol was analyzed in full-blood with a LC-MS method and/or in plasma with a Cobas c 111 analyzer (Roche Diagnostics). Results: The optimal p.o. dose of paracetamol was found to be 50 mg/kg in both rats and mice. As expected, carbachol increased blood levels of paracetamol compared to vehicle, suggesting that gastric emptying was stimulated. In contrast, atropine inhibited gastric emptying, as evidenced by lower plasma paracetamol levels relative to vehicle. In rats, GLP-1 inhibited gastric emptying as expected, and the LC-MS method was more sensitive than the Cobas Analyzer assay. Conclusion: We have confirmed the validity of the paracetamol method in mice using atropine and carbachol, and in rats using GLP-1. Furthermore, we have developed a LC-MS method that is more sensitive and requires less blood than the Cobas Analyzer assay. Thus, with the LC-MS assay it is possible to reduce the volume of blood and/or reduce the number of animals in future studies.

PA12 A modified model for intra colonic dosing in rodents.

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Colon targeted drug delivery is gaining interest for gastrointestinal related diseases (colorectal cancer and inflammatory bowel disease). Local colon targeting increases efficacy by increasing target organ exposure and safety by lowering systemic or non-target tissue exposure. Modelling approaches are at this stage not mature enough to be able to predict the intra colonic profile. There is a need for a screening approach to evaluate colon and blood exposure and colon to blood ratio in vivo.
In this respect we developed and optimized models for acute and chronic intra colonic dosing in rodents (mouse and rat).Intra colonic dosing can be performed by dosing in the colon via a needle or via a catheter. In most intra colonic dosing models an indwelling PE catheter is placed directly in to the colon and externalized via the neck. Infection due to leakage of the colon is often seen when the catheter is directly inserted in the colon. We refined the intra colonic model by implanting a Penny port (a small, light-weight access port) in the abdomen. The catheter connected to the port is advanced from the caecum into the proximal part of the colon. The catheter is fixed to the caecum to avoid leaking of the gut into the abdomen and leaving the colon intact. Thus infections can be avoided and survival of the animals prolonged. The model allows multiple dosing in the colon, reuse of the animals (cross over design) and the comparing of the exposure in plasma and tissue after intra colonic dosing versus oral administration.

PA13 Mouse Papillomavirus (MmuPV) Infection in Mice: A Model for Head and Neck Cancers in Humans

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Papillomaviruses (PVs) induce papillomas, premalignant lesions, and carcinomas in a wide variety of species. PVs are classified by host, tissue tropism and genomic diversity. A laboratory mouse papillomavirus, MmuPV1 (formerly MusPV), was horizontally transmitted in an inbred colony. Immunocompetent, alopecic, S/RV/Cri-ba/ba (bare) mice developed small papillomas at injection sites that regressed. NMRI-Foxn1nu and B6. Cg-Foxn1nu, but not NU/J-Foxn1nu, mice were susceptible to MmuPV1 infection.
Papillomaviruses (PVs) induce papillomas, premalignant lesions, and carcinomas in a wide variety of species. PVs are classified first based on their host and tissue tropism and then their genomic diversities. A laboratory mouse papillomavirus, MmuPV1 (formerly MusPV), was horizontally transmitted within an inbred colony of NMRI-Foxn1nu/Foxn1nu (nude; T cell deficient) mice for an unknown period of time. A ground-up, filtered papilloma inoculum was not capable of infecting C57BL/6J wild-type mice; however, immunocompetent, alopecic, S/RV/Cri-ba/ba (bare) mice developed small papillomas at injection sites that regressed. NMRI-Foxn1nu and B6. Cg-Foxn1nu, but not NU/J-Foxn1nu, mice were susceptible to MmuPV1 infection.B6 congenic strains, but not other congenic strains carrying the same allelic mutations, lacking B- and T-cells, but not B-cells alone, were susceptible to infection, indicating that mouse strain and T-cell deficiency are critical to tumor formation. Although lesions initially observed were exophytic papillomas around the muzzle, exophytic papillomas on the tail and condylomas of the vaginal lining could be induced either by separate scarification or simultaneous scarification of MmuPV1 at all four sites. On the dorsal skin, locally invasive, poorly differentiated tumors developed with features similar to human trichoblastomas. Transcriptome analysis revealed significant differences between the normal skin in these anatomic sites and in papillomas versus trichoblastomas. The primarily dysregulated genes involved molecular pathways associated with cancer, cellular development, cellular growth and proliferation, cell morphology, and connective tissue development and function. Although tricheoepitheliomas are benign, aggressive tumors, few of the genes commonly associated with basal cell carcinoma or squamous cells carcinoma were significantly dysregulated.

PA14 Evaluation of the impact of group housing in mice instrumented with brain eeg electrodes

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The goal of the present study was to assess the possibility of avoiding single housing in C57Bl/6J mice with or without EEG implants, and to evaluate the potential impact on the quality of EEG signals and on the health and welfare of the animals (stress, dominance...). Mice were first submitted to the SSSE model and developed the so-called status epilepticus (SE) which was stopped by diazepam administration. Few months later, they were distributed in different cages to perform group housing.
Electroencephalography (EEG) is the main diagnostic tool for epilepsy and sleep disorders. Electrodes implantation in rodent brain is widely used in preclinical research in epilepsy models as such kindling, self-sustained status epilepticus (SSSE) and also to monitor sleep cycles through quantified EEG. The goal of the present study was to assess the possibility of avoiding single housing in C57Bl/6J mice with or without EEG electrode implants, and to evaluate the potential impact on the quality of EEG signals and on the health and welfare of the animals (stress, dominance...). Mice were first submitted to the SSSE model and developed the so-called status epilepticus (SE) which was stopped by diazepam administration. Few months later, they were distributed in different cages to perform group housing.Five pairs of mice were housed in five conventional type II Makrolon cages. Different possibilities were assessed, namely 2 "naïve" mice (no implantation), 2 implanted non-stimulated mice ("sham"), 2 implanted stimulated mice ("SE"), one "naïve" and one "sham", one "sham" and one "SE". For comparison, 10 C57Bl/6J instrumented mice (5 "sham" and 5 "SE") were single-housed in the same husbandry. Pictures and clinical observations were compared at different time-points: on the first day, after one month of single or group housing, and at the end of the 2 months of observation.EEG traces were recorded on the instrumented mice at the beginning and the end of the same period of time. We conclude that EEG recordings before and after 2 months of group housing have essentially identical quality compared to single-housed mice, but 40% of the group-housed mice showed injuries and scars due to aggression This was more severe in "sham" mice. Consequently, group housing negatively affects well-being of the animals and increases the risks for damage of electrode implants. Therefore, in the context of animal welfare, we do not recommend group housing of EEG-instrumented mice.

PA15 Effect of immune deprivation of IL-2 on tumor growth in melanoma MB16F10 nude NMRI mice.

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The discovery of the ability of the immune system and specifically of T cells to recognize tumor antigens has been one of the fundamental pillars for the development of strategies to manipulate the immune system to treat cancer patients. One of the main assumptions indicate that a failure in the production of CD4 + CD25 + regulatory T cells causes the onset of autoimmune signs in the absence of IL-2, so that the main activity is not redundant of this cytokine talk it is to maintain tolerance.
Fifteen NMRI nu/nu mice were used. They were kept in ventilated rack (TECNIPLAST), and were subcutaneously inoculated with 200 000 MB16F10 cells. Animals were divided into 4 experimental groups. Animals received IL-2 (Group I); anti-IL-2 monoclonal antibody (Group II); the combination of both products (Group III); and a control group (Group IV), the kinetics of tumor growth was assessed, at 21 days euthanasia was practiced, lymphocyte subsets were counted from CD3, CD4, CD8 and B220 in spleen and tumor markers. In this paper we have the objective of evaluating the effect of immune deprivation of IL-2 on tumor growth in melanoma MB16F10 nude NMRI mice.No significant differences in tumor growth or in populations analyzed were found. We concluded that the administration of IL-2 and / or administration of anti-IL-2 antibodies have no direct effect on tumor growth in NMRI nude mice, so the increased growth of allogeneic tumor in these animals is an effect mediated by T cells.

PA16 Protective effect of Glycyrrhiza glabra root extract on bone mineral density of ovariectomized rats

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Postmenopausal osteoporosis affects women worldwide. Pharmacological intervention is considered as the optimal prevention path. Glycyrrhiza glabra, also referred to as liquorice, is a phytoestrogen-rich plant historically utilized for its roots (1). Phytoestrogens are plant-derived phenolic compounds exerting osteoprotective properties. Previous research confirmed the presence of phytoestrogens and other constituents isolated from G. glabra roots with estrogenic-like activity (2).
Our aim was to investigate the potential protective effect of the methanolic extract of the roots of G. glabra (G) on bone mineral density (BMD) and femoral bone strength of the ovariectomized (OVX) rat model of osteoporosis. Material and Methods: Thirty 10-month-old Wistar rats were randomly separated into three groups of ten; Control, OVX and OVX plus G (OVX+G) in their drinking water. Total and proximal (trabecular) tibial BMD was measured in all groups before ovariectomies (baseline) and after 3 and 6 months post-surgery. Three-point-bending of the femurs and uterine weight were examined after euthanasia at the end of the study. Results: No considerable difference was noted in BMD percentage change of total tibia from baseline to 3 months between groups Control and OVX+G (+5.31%±4.75 and +3.30%±6.31 respectively, p=NS), and of proximal tibia accordingly (+5.58%±6.92 and +2.61%±13.62, p=NS) demonstrating a strong osteoprotective effect. There was significant difference in BMD percentage change of total tibia from baseline to 6 months between groups OVX and OVX+G (-13.03%±5.11 and -0.84%±7.63 respectively, p<0.0005), and of proximal tibia accordingly (-27.9%±3.69 and -0.81%±14.85 respectively, p<0.0005), confirming the protective effect of G. glabra extract in preserving the BMD of the OVX+G group. Three-point-bending did not reveal any statistically significant difference between groups OVX and OVX+G. Uterine weights of the OVX+G group ranged between the other two groups with no statistically significant difference. NS=non-significant.Discussion: The fact that in vitro and in vivo studies showed that liquorice root extract exhibits varying degrees of estrogen receptor (ER) agonism in different tissues (2) led us to further investigate the estrogen-like activity on bone in vivo. It demonstrated the beneficial effect of G. glabra extract (G) on the BMD of the most used rat model for the study of human osteoporosis (3). Significant BMD protection demonstrated in trabecular bone measurement was not accompanied by bone strength protection, probably due to the test being conducted in the tibial shaft which is cortical bone-dominated and late in showing treatment effect. Conclusions: Glycyrrhiza glabra root extract notably protected tibial BMD loss in OVX+G rats in comparison to OVX rats, but did not improve biomechanical strength, which renders this field still explorable.

PA17 Use of historical control data to reduce group size in in vivo studies: an example from a test of visuo-spatial recognition memory

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Cognitive functions are often impaired in neurological illnesses like Alzheimer's disease. While molecular aspects can be modelled in vitro, investigation of higher brain functions and the effect of experimental drugs requires testing in live animals. However, behavioural data are often subject to considerable variability. The use of historical control data and appropriate statistical methods can help to generate meaningful data while minimising the number of animals used.
Materials and methodsThe set-up consisted of two arms with distinct visual cues, connected to a central area to form a V-shaped maze. Male Long Evans rats were first given 5 min to explore one of the arms. Then the second arm was opened giving rats a choice to explore the new or the previously visited familiar arm. Exploration was recorded as time spent in each arm using video analysis software. Since exploration of a novel environment is an innate behaviour of rodents, an increase in time spent in the novel arm relative to the familiar arm is expected. This is expressed as the Discrimination Index (DI) and is interpreted as a measure of recognition memory. This behaviour can be disrupted by treatment with an NMDA receptor antagonist, e.g. phencyclidine (PCP, 0.75mg/kg, SC, 30 min prior to testing).In routine testing, animals are randomly assigned to the following treatments: positive control (vehicle), negative control (disruptor) or test treatment (disruptor + test compound, up to three doses/compounds), and DI is measured. Historical data are available for positive and negative control from multiple experiments. Between-study variability for the effect of control treatments was investigated using random effect meta-analysis (Ref 1,2). Meta-analysis results were used as input for simulations to determine the relationship between statistical power (to detect a significant increase of recognition with test treatment versus negative control - α=0.05, one-tailed hypothesis) and sample size (max N=60, multifold of 4 per treatment). Several scenario's for experimental design (unbalanced and balanced), effect sizes and variability were considered. Historical disruptor control data were included in statistical analyses by means of mixed effect models. ResultsThe meta-analysis indicated that the difference in recognition memory between positive and negative controls was consistent across historical experiments, but within-experiment variability was considerable.Inclusion of historical disruptor control data in the analysis considerably increased the power to detect an effect of test compound. Reasonable power could be obtained

with fewer animals per experiment when historical negative control data were included than when not. Discussion and conclusionThese results illustrate that incorporating historical control data in the design and analysis of routinely performed animal in vivo experiments allow a more ethically justified use of animals.

PA18 Refinement of Vascular Catheterisation in Rats: Use and patency of PinPort™

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Placement of a vascular catheter facilitates blood withdrawal and/or administration of compounds in animal models is a refinement to frequent handling and repeated vascular punctures. Connection to the exteriorised portion of catheter can be enhanced with the use of an external access port, i.e. PinPort™ in CD®IGS rats. We conclude that PinPort™ can remain in place for up to 30 days (low usage) and with a guaranteed vascular catheter patency for 15 days, if associated with frequent sampling.
MATERIAL Rats CD®IGS (SD) Charles River Laboratory France, 250-275g male were born on site, had SPF/VAF Plus heath status. Following pre-operative acclimatisation, rats were anaesthetised with intra-peritoneal (IP) injection of ketamine 43mg/kg and xylazine 8.7mg/kg. Following surgical field preparation vascular catheters were implanted, through femoral vein. Catheter was secured and exteriorized in the inter-scapular region. The polyurethane catheter was stitched to the skin with prolene 4-0 and the PinPort™ was fixed securely with a drop of Loctite® glue. Anaesthesia was reversed with SC injection of atipamezole and analgesic buprenorphine 0.05mg/kg SC given on day of the surgery, day one post-op. Catheters were flushed with NaCl 0.9% and locked with heparin (500USP/ml) glycerol Cath-LocHGSH. Rats were housed individually in surgical facility which is accredited by the French Ministry and AAALAC International. METHODS In the Pilot study designed to assess patency with regular usage, catheters were flushed from Day +5 after surgery, then every day until the functionality was lost. In Validation study the aim was to assess patency with intensive usage. The attachment in the inter scapular area was supported by an additional non-absorbable suture around the catheter, between the PinPort™ and the distal stop. Catheters were flushed 3 times a day, for 15 consecutive days (except during weekend when equipment handled to simulate its use), starting from Day +4 after surgery. All animals with functional catheters were euthanised on day 37 (1st study) or day 18 (2nd study) and assessed macroscopically for the presence of signs of infection or inflammation. RESULTS AND CONCLUSIONS In the Pilot study catheters in 9/12 rats (75%), were patent during the 37 days of the study. The 3 rats were humanely killed due to detachment of the catheter in the inter-scapula region. In the Validation study catheters of 12/12 rats (100%) had intact inter-scapular fixation and remained patent during 15 day of intense usage. The autopsies performed on all these animals showed no evidence of macroscopic abnormalities. We conclude that PinPort™ can remain in place for up to 30 days (low usage) and with a guaranteed vascular catheter patency for up to 15 days, if associated with frequent use.

PA19 Assessment of different heat sources for blood sampling in female C57BL/6 and BALB/c mice

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In this study different sources of heat to dilate mouse veins for blood sampling were tested: an infrared lamp (LI), a standard warming-cabinet (AC) and a mini warming-cabinet (MT). The objective was to assess which one was better with regard to both animal welfare and sampling technique. Materials and methods. Female C57BL/6 and BALB/c were dosed and sampled at 20, 40 and 60 minutes from the tail veins after warming for 10 minutes. At each time point, the number of needle punctures, the effort and the time needed to obtain the sample were recorded blindly. Body weight was recorded before dosing and after the last sample. Then animals were euthanized and blood sampled for hematology and biochemistry analysis and plasma corticosterone determination. Controls included a non-handled group (basal control) and a group that was dosed and sampled but not warmed (procedure control). Additionally, the technician was asked to guess the heat source, blindly and with earplugs, and to assess the usability of each of the warming sources. Animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. Results. The complete pharmacokinetic procedure could be considered relatively stressful for mice, as shown by body weight loss, plasma corticosterone rise and the alteration of some hematological and biochemical parameters. Mice sampled without warming did not show lower stress and warming was necessary to obtain rapidly a sample of sufficient volume. The LI was the source that needed less needle punctures, effort and time to obtain the sample, although the difference was only significant for the time needed in BALB/c (two-way ANOVA, $p=0.0323$). On the other hand, the LI produced the highest rise in corticosterone in the C57BL/6 strain (ANOVA, $p<0.0001$), and the lower values in erythrocyte count, hemoglobin and hematocrit in BALB/c mice (not statistically significant). The technician was able to guess the source of heat in 45.8-50% of the mice; the percentage of success was higher for the LI and lower for the AC. The MT was considered the more practical warming source due to its reduced size, short time to achieve set temperature, and easy handling and access to animals. Conclusion. The LI was the best source for warming mice as less time was needed to obtain a sample. However, it was also the heat source that produced higher levels of stress. Therefore, we recommend that the AC and MT are used preferentially over the LI, except in situations in which, due to the specific characteristics of the animals or the drugs dosed, a more efficient warming is necessary. Additionally, the MT was preferred over the AC by technicians due to its better usability.

PA20 Proof of pluripotency in experimental induced teratomas and embryoid bodies by immunohistochemistry

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In stem cell research, pluripotency of stem cell lines is proven by the in vivo teratoma assay. Putatively pluripotent cells are injected into immunodeficient mice to form teratomas. These tumors contain tissue of the embryonic germ layers, i.e. endoderm, mesoderm, and ectoderm. Aim of the study is to establish an immunohistochemical antibody panel to facilitate diagnosis of all germ layers especially in immature teratomas and also embryoid bodies as an alternative in vitro cultivation method.
MATERIAL and METHODS Three specific murine induced pluripotent stem cell lines (iPSC) were injected subcutaneously into NSG mice. Additionally, four iPSC lines of the non-human primate Callithrix jacchus were injected under the kidney capsule of NSG mice in order to form teratomas. Alternatively, the same iPSC lines were also grown in suspension culture to show their differentiation potential in vitro. Resulting tumors and embryoid bodies (EBs) underwent histopathological analysis. In addition to H&E staining to get an overview of the structures, immunohistochemistry was performed of markers representing all embryonic germ layers. As an example, Cytokeratin 18 is used for labelling endodermal tissue, whereas muscle as mesodermal derivative was detected by desmin. Beta-III-tubulin and neurofilament 160 label neural tissues, i.e. ectodermal structures. RESULTS Non-human primate iPSCs formed less differentiated tumors with blast-like cells in all cases. On the other hand, teratomas derived from murine stem cells mostly contained mature tissues like bone, cartilage, intestinal, respiratory and squamous epithelium as well as neurons and different types of muscle. Immunohistochemical proof of pluripotency was performed successfully in 57 of 59 murine teratomas and in every teratoma derived from stem cells of Callithrix jacchus. All in vitro cultivated iPSCs formed embryoid bodies with a morphologically low differentiation. Some showed cystic areas and necrosis, others were of solid cell accumulation. However, with the applied immunohistochemical antibody panel all three embryonic germ layers stained positive, reflecting the results from teratoma formation. CONCLUSION In well differentiated teratomas H&E staining is sufficient to diagnose the three embryonic germ layers. In tumors with a morphologically undifferentiated state, immunohistochemistry enables the proof of pluripotency. In this study, in all iPSC lines proven to be pluripotent with the in vivo teratoma assay, pluripotency could also be shown in the in vitro cultivation using the established immunohistochemical antibody panel. The possibility of replacing the in vivo teratoma assays should be considered in studies where appropriate.

PA21 Using alternative in vitro methods to study reversion of antibiotic resistance

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Researches directed to find compounds, affecting the antibiotic sensitivity of resistant mutants are under investigation in the Scientific center for anti-infectious drugs. Previously, it was proved, that FS-1, a new drug, developed in the Scientific center for anti-infectious drugs, caused the reversion of resistance mutants (Ilin A.I. et al. 2015).
Pro-inflammatory cytokines were suggested as one of biological markers of antibiotic susceptibility changes, associated with bacterial pathogen-associated molecular patterns (PAMP). For this purpose technique to study immunopathogenesis on human peripheral blood mononuclear cells (PBMCs) model was adapted and introduced into practice for the evaluation of reversion of antibiotic resistance (Kapoor N. et al. 2013). In vitro studies were performed under Good Laboratory Practice (GLP), which ensured control and quality of those researches. Isolation of PBMCs was performed according to the density gradient separation method (Boyum A. 1968). Resistant and sensitive Escherichia coli (E. Coli) ATCC 8739 cultures were used as a pathogen model. Activity and specificity of the system were evaluated by microorganism's PAMP changes before and after FS-1 drug exposure. Pro-inflammatory cytokines IL-1b, IL-6 and TNF-alpha were served as biological markers. Comparing to sensitive E. Coli culture, PAMPs of resistant E. Coli mutants induced 2.3 times higher production of IL-1b, 12.9 times higher production of IL-6, and 5.7 times higher production of TNF-alpha. It was demonstrated, that PAMPs of resistant E. Coli culture induced the next amount of products: IL-1b – 5.2 pg/ml, IL-6 – 82.3 pg/ml, TNF-alpha – 46.6 pg/ml. Treatment of resistant E. Coli mutants with the combination of FS-1 drug with ampicillin in a concentration of 1/8 of MIC, resulted in increased production of IL-1b to 236.4 pg/ml, IL- 6 to 374.3 pg/ml, TNF-alpha to 852.9 pg/ml.In general, this alternative model to study the reversion of antibiotic resistance, based on PAMP changes of resistant bacteria after FS-1 treatment, can be further used in experimental studies.

PA22 Functional analysis of genetical changes in the LEW.1AR1-iddm rat – an animal model for Diabetes type I

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The LEW.1AR-iddm rat arose through a spontaneous mutation within the inbred strain LEW.1AR1. The mutation leads to a variable T cell frequency in the peripheral blood of the animals in which 60% develop autoimmune diabetes. Two base pair exchanges are unique for the LEW.1AR1-iddm rat and are supposed to be the result of a natural mutation in LEW.1AR1. One mutation is in Dock8 the other one in a predicted C/EBPβ binding site at position BNRnor_5.0 242,319,497.
Materials and Methods: Both mutations could be separated by generating (LEW.1AR1xLEW.1AR1-iddm) F2 rats. Blood glucose measurements, flow cytometry of the peripheral blood, thymus weight, histology and immunohistochemistry were determined to analyze the different phenotypes. Results: The point mutation in Dock8 causes diabetes but only in 27 % of the animals and age at manifestation shifts to older ages. In these rats T cell frequencies in the peripheral blood are decreased to even lower numbers than in LEW.1AR1-iddm. In addition regulatory T cells are also reduced. Histological experiments revealed that pancreas infiltration can be scored with the same score used for LEW.1AR1-iddm rats. Thymi morphology shows more vessels in the medulla when compared to LEW.1AR1. The point mutation in the predicted C/EBPβ binding site seems to

be responsible for the variable T cells frequencies in the peripheral blood. This variability was also seen in LEW.1AR1-iddm rats. Regulatory T cells are comparable to LEW.1AR1 cells. Rats carrying only the mutation in the C/EBPβ binding site show also infiltrating mononuclear cells into islets but don´t develop diabetes. Infiltration is far more in the beginning and looks like about to start. Therefore pancreas probes are not scorable yet. Thymi morphology shows smaller medulla when compared to the LEW.1AR1 coisogenic control strain. Conclusion: In this study we found out that both point mutations have a synergistically effect on the pathogenesis of the LEW.1AR1-iddm rat including pancreas infiltration, Thymus morphology, T cell differences and diabetes development.

PA23 Refinement of serial blood sampling in cannulated rats with the use of the pinport®

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Pharmacokinetic (PK) studies are regularly carried out in rats to determine the pharmacokinetic properties of new chemical entities in order to progress the best compounds to the development of new treatments. Rat PK studies are performed in surgically cannulated animals for serial blood sampling as recommended by FELASA and the NC3Rs. We have refined the surgical model by modifying the externalization system and the access to the catheter with the use of the pinport.

Male SD rats were surgically cannulated in the right jugular; the catheter was tunneled under the skin and externalized through the area between the scapula. Three different systems were tested in consecutive order:1) Dacron button (DB): externalization through a silicone tube held in place subcutaneously by a Dacron mesh. The catheter was protected with a metallic spring with a swivel. The catheter was accessed for blood sampling by using a needle port. Animals are housed individually in modified cages with limited environmental enrichment to avoid tangling with the spring.2) Harness: externalization through the skin of the back. The metallic spring was held in place with a silicone harness. The tether system and the housing were identical to the DB.3) Pinport: only a small portion of the catheter was externalized through the back; the catheter was glued to the skin to prevent the rat from pulling. Animals were housed in standard cages with the standard enrichment. Blood was sampled from the catheter by using a miniature port (Pinport). Animals were assessed for 1) infection 2) well-being: score for body posture, appearance and reaction to handling, 3) catheter patency, 4) body weight, and 5) food and water consumption.Infection was present in the subcutaneous area where the DB was inserted in 66.7% of the rats; bacteremia appeared in 41.7%. DB animals often showed a hunched posture, minor piloerection, and vocalizations when handled (audible and 22KHz vocalizations). Harness animals had no infection, but often showed a hunched posture, marked piloerection and vocalizations when handled. Additionally, some animals showed inflammation of the neck and sores around the forelegs due to the pressure of the harness. With the Pinport method no infection was observed, and body weight loss and the days needed to recover pre-surgery weight were reduced. Pinport catheter patency was the highest (93%), compared to the harness group (83%) and the DB group (58%). Pinport rats also showed a normal posture, no piloerection and extremely infrequent 22KHz vocalizations. Animals were free to move in the cage and could use the standard enrichment. The Pinport system was also the preferred one by technicians. The pinport system is the best option with regard to the welfare of animals and the quality of the model, and thus we have adopted this system as routine for our PK studies.

PA24 Use of the reference change value to routine hematology and biochemistry test results in healthy laboratory cats

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In toxicology studies, interpretation of routine hematology and biochemistry is based on comparison of results obtained in test animals with controls or reference intervals. This masks the intraindividual variability. Reference change value (RCV), i.e. highest difference possibly due to intraindividual and analytical variability, is a more effective way to monitor individual changes over time. The aim of this study was to determine RCV of hematology and biochemistry variables in laboratory cats.

Materials and methods: Fourteen overnight-fasted laboratory cats were sampled 7 times over 3 months and analysed for routine hematology and biochemistry. Analytical, intraindividual and interindividual coefficients of variation (CVa, CVi and CVg respectively) were estimated to calculate the RCV = 2.77 (CVi2 + CVa2)0.5. Results: RCV were lower than 30% for red blood cell (RBC) count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), albumin, cholesterol, creatinine, total proteins, triglycerides, urea, alkaline phosphatase (ALP), calcium, chloride, carbon dioxide, magnesium, inorganic phosphate, potassium and sodium. It ranged from 35% to 70% for white blood cell, neutrophil, lymphocyte, monocyte, eosinophil and platelet counts, glucose, alanine aminotransferase, aspartate aminotransferase and iron. It was higher for reticulocyte count (99%) and creatine kinase (143%). Discussion and conclusions: Analytical, intra- and inter-individual variabilities are confounded in population-based reference intervals and control groups. Being a more sensitive index to detect “abnormal” changes over time in a given individual, the RCV also allows to reduce the number of animals used to increase the power of statistical tests but it can only be performed in animals large enough to allow repeated sampling, such as cats. This study showed that low RCV (≤ 30%) would be preferable to monitor red blood cells variables and numerous biochemical analytes in laboratory cats. The RCVs determined in this study provide an efficient tool for monitoring hematological and biochemical variables in individual healthy laboratory cat. Moreover, as the analytical variability is generally much lower than the intraindividual variability and is almost identical between laboratories the preceding RCV can be transferred to other laboratories working with laboratory cats.

PA25 Characterization of the hairless NOD.SCID mouse model (SHrN™)

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The SHrN™ hairless NOD.SCID mouse was developed to establish a triple-immunodeficient hairless mouse model. The non-obese diabetic (NOD) mouse carrying the Prkdcscid mutation (NOD.SCID mouse) is an established xenograft model for humanization, oncology, and immunology research. As SCID mice are haired, shaving is necessary to facilitate tumor observation and evaluation. However, shaving animals does not prevent autofluorescence of hair follicles while imaging.

Flow cytometric analysis confirmed that the SHrN™ mice were nearly completely lacking T-cells and B-cells (Figure 1). CD19, a common B-cell lineage marker, showed expected results; both strains were nearly devoid of mature B-cells. The SHrN™ mice had fewer lymphocytes overall than NOD.SCID mice; in particular NK cells and dendritic cells in the SHrN™ were markedly decreased in comparison to the NOD.SCID. Significant differences (P0.05) were reported between the T-, NK-, and NKT-cell sub-populations as well as the dendritic cells of both strains. All IgG measurements for SHrN™ mice were <6.25µg/mL, or below the threshold for leakiness in SCID models.Lymph node and spleen histology were completed on these mice; analysis of bone marrow sections was completed but no differences were noted between strains. The lymph nodes and splenic white pulp are void of cells with typical lymphocyte morphology (e.g. condensed nucleus, scant cytoplasm). Reticular support cells, dendritic cells, and macrophages remained, and based on flow cytometry data, NK cells were also present. There were varying degrees of hematopoiesis in the splenic red pulp. Future assessment will include immunohistochemistry evaluation on these sections using the various markers used for flow cytometry. The JEKO-1 tumor cell line can be successfully transplanted into athymic nude mice whether or not they are irradiated prior to inoculation, though initial proliferation is slow. In this study, the nude mice had approximately 25–27 days lag time to log-phase growth, whereas the SHrN™ mouse showed rapid and vigorous growth. Indeed, all SHrN™ mice developed tumors within 14 days post inoculation. The KARPAS-299 cell line will not grow in athymic mice without whole body irradiation. In contrast, the SHrN™ model exhibited robust growth with subcutaneous injection without irradiation.In summary, these data demonstrate that new SHrN™ hairless NOD.SCID mouse model has a severe deficit in B- and T-cells and a marked decrease in both NK cells and dendritic cells as compared to a standard NOD.SCID mouse. The increased immunodeficiency of this model could result in a much more efficacious model for rapid tumor uptake or for engraftment of human hematopoietic cells. In addition, the lack of hair in this model both reduces animal preparation time for tumor cell inoculation and increases tumor imaging clarity.

PA26 Osteoprotective ability of the methanolic extract of Iris unguicularis subsp. cretensis on ovariectomized rats

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Osteoporosis is a degenerative bone disease that affects millions of women, especially after menopause. Nowadays, there are concerns about hormone replacement therapy (HRT) due to its side effects. Therapeutic alternatives are sought in order to address these concerns [1]. Moreover, evidence for the effect of herbs on bone mineral density has been proved [2] and especially among Iris species there are in vitro studies that provide potential osteoprotective properties [3].
The aim of this study was to investigate the potential osteoprotective effect of the methanolic extract of Iris unguicularis subsp. cretensis roots (IUCM), on bone mineral density (BMD) on the mature ovariectomized (OVX) rat model of osteoporosis. Material and Methods: Twenty eight 10 months old Wistar rats were randomly assigned into three groups. Group A (n=8) was served as Control, Group B (n=10) was OVX and Group C (n=10) was OVX and treated with IUCM (200mg/kg/day) through drinking water. The IUCM administration started immediately after ovariectomy, till the end of 6 months. BMD of the proximal and total tibia was measured at the beginning (baseline) and 3 and 6 months after ovariectomy using Dual Energy X-ray Absorptiometry. Organs were harvested and weighed after euthanasia and three-point bending of right and left femurs was performed. Results: Total tibia median percentage changes from baseline were 5.31% and 11.59% for Group A, -8.87% and -13.03% for Group B, -3.03% and -5.72% for Group C, at 3 and 6 months respectively. Pairwise comparison revealed statistical significant differences between all groups. For proximal tibia metaphysis BMD median percentage changes from baseline were 5.58% and 9.84% for Group A, -18.42% and -27.86% for Group B, -14.02% and -19.69% for Group C, at 3 and 6 months respectively. Statistical significant differences were observed between Group A and Group B, as well as Group C at 3 and 6 months and between Group B and Group C at 6 months measurement. Three-point bending revealed no significant differences between groups. No significant differences were observed in organs weight between groups, except for total fat which was significant higher in Group B and Group C compared with Group A. Uterus weight was significant lower in Group B (0.37g) and Group C (0.2g) compared with Control (0.63g) and uterus weight of Group C tended to be lower compared with Group B. Statistical significance was defined as $p<0.05$. Conclusions: Iris extract was able to protect total and proximal tibia from bone loss, as revealed from the comparison of BMD between Group B and C, especially after 6 months administration, but this effect wasn't so potent, when compared to control Group. This effect of IUCM was not accompanied with biomechanical strength protection and needs further investigation. IUCM administration did not affect the wet weight of the animals' uteri, which were atrophic and of similar weight as those of the non-treated OVX rats.

PA27 Preclinical imaging study of mouse models of dementia

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Dementia is defined as a chronic mental disorder caused by brain disease or injury. Clinical symptom includes memory disorders, personality changes, and impaired reasoning. The most common type of dementia is Alzheimer's disease and the second type is vascular dementia. To develop dementia medicine, preclinical animal models are critical for drug testing. In vivo imaging techniques are reliable and efficient tools for testing drug efficacy.
Alzheimer's disease is characterised with amyloid plaque accumulation in the brain.5XFAD transgenic mouse over-expressed with 5 Familial Alzheimer's Disease (FAD) mutations was used to establish imaging protocol for testing Alzheimer's disease. A mouse model of vascular dementia was produced with bilateral common carotid artery stenosis (BCAS) surgery technique. Apolipoprotein E (APOE) knock-out mouse was initially fed with 6 weeks of high fat diet and underwent BCAS surgery.Brain pathology was examined with magnetic resonance imaging (MRI) technique and cognitive impairment was monitored with behaviour tests in dementia mice. To monitor morphological changes in the brain, 5XFAD mouse and APOE knock-out mouse with BCAS were repeatedly scanned with Bruker 9.4T MRI. Specifically, the mouse brain was monitored with T1, T2, diffusion tensor imaging protocol. In addition, BCAS mouse was scanned with angiography before and after surgery to find vascular damage. Cognitive deficit was evaluated with open field test, Morris water maze test and novel object recognition test.Neuronal atrophy (shown in hypo-intensity of T2 MRI images) appeared in the hippocampus of 5XFAD mouse over 6 months. BCAS in APOE knock-out mice also developed hippocampal damages 2~3 months after surgery. BCAS also produced vascular damages monitored with MR angiography compared to wild type mice. Together with brain damages, dementia mice showed significant memory impairment in behaviour tests. Mouse models of dementia could be systemically examined both by brain pathology and cognition using MRI and behavioural tests. Combinations of in vivo imaging and behavioural test provide effective tools for testing drug candidate to treat dementia.

PA28 Evaluation of a prototype HD-XG telemetry implant for real-time continuous glucose monitoring in mice

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Blood glucose measurement is routine during preclinical diabetes and metabolic research. For glucometer and test strip measures blood samples have to be drawn. Data quality strongly depends on the animals' stress level. Significant events might get lost due to gaps between samples. The HD-XG is an implantable telemetry device providing continuous real-time measurements of blood glucose, temperature, and activity in rats. The present study evaluates the use of a novel HD-XG prototype for mice.
Materials & Methods8 male 3 months old C57Bl/6 mice were implanted with HD-XG prototypes for mice (DSI, St. Paul). The glucose sensor tip was inserted into the left carotid artery and advanced into the aortic arch. The transmitter body was placed subcutaneously at the right flank. All animals were housed under intensive care (analgesic, fluid, warm environment) for the first postoperative week. Data acquisition started with the first calibration at day 3 post surgery and data were collected continuously until the end of the 3rd week after device implantation. All sensors were calibrated with glucometer data by taking blood samples from the tail tip. For initial calibration a two point IPGTT was performed (2.7g glucose/kg bodyweight). Thereafter, blood samples were taken twice per week for calibration. ResultsAll mice showed unremarkable wound healing with a transitory mild body weight loss within the first postoperative week. A clear day-night rhythm was observed for blood glucose, activity and body temperature in all mice with higher average blood glucose levels in concurrence with higher body temperature and activity levels during the active phase (night). Even though the average glucose levels were lower during the light period excursions which went above the dark period excursions could be observed. Blood glucose levels were highly individual and constantly fluctuated depending on multiple factors (e.g. activity, meals). During the IPGTT a significant increase in blood glucose levels was observed within 15 minutes after IP glucose injection but again individual differences could be observed between mice. All animals returned to baseline levels within 90 minutes.Discussion & ConclusionThe HD-XG mouse prototype offers real-time blood glucose measurement in conscious freely moving mice. It continuously detects changes in blood glucose levels and the obtained data provide quantitative insight into glucose metabolism and homeostasis. A single measured glucose level was not indicative of a period average. The concurrent measurement of the animal's activity level and body temperature offers valuable additional information for the interpretation of blood glucose level's changes as well as animal wellbeing in general. Blood sampling stresses mice as documented by increased activity and body temperature levels after sampling. The use of continuous glucose telemetry allows for a reduction in sampling thus reducing animal stress during the experiment.

PA29 Histological lesions associated with pressure sensing catheters implanted for arterial blood pressure measurement in mice

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Radiotelemetry is thought to be the gold standard for long-term blood pressure measurements in rodents. In mice it is preferred to place the pressure sensing catheter tip into the aortic arch via the carotid artery. Whilst it is well known that venous blood sampling catheters can lead to clot formation, tissue irritation and inflammation, no such information is available for arterial catheters. The present study evaluates possible lesions associated with blood pressure catheters.
Materials & Methods Twenty 3 month-old male C57BL/6 mice were implanted with PA-C10 telemetric devices (DSI, St. Pauls). The pressure sensing catheter was inserted into the left carotid artery, advanced into the aortic arch and fixed by sutures. The transmitter body was placed SC in the right flank. All animals were housed under intensive care (analgesics, fluids, warm environment) for the first postoperative week. Mice were fed with a standard rodent diet (Kliba Nafag 3436) except during the last 2 weeks in which they were fed a high salt diet (Kliba Nafag 3436, 8% NaCl). Blood pressure was measured until the end of the 7th postoperative week, when all mice were sacrificed and underwent a thorough postmortem analysis. Results Postoperative recovery was uneventful in all mice and characterized by unremarkable wound healing with transitory mild body weight loss, which recovered by the first week after surgery. Necropsy confirmed that all catheters were placed with approximately 2mm of the catheter tip entering the aortic arch. Histology of the aortic arch revealed the presence of locally proliferative lesions at the level of the catheter tip in the majority of animals, represented by remodeling of the aortic wall, cartilage metaplasia, partial luminal occlusion and, occasionally, by thrombosis. There was also a severe chronic fibrosing and proliferative arteritis narrowing the lumen of the carotid artery with the indwelling catheter. No correlation could be drawn between blood pressure data and the type or severity of lesions. However, ultrasound examination demonstrated that even a correctly placed catheter tip creates clearly visible turbulences within the blood flow and might occasionally strike the aortic wall due to its rhythmic movement caused by pulsation. Discussion & Conclusion Focal proliferative reactions with variable severity and location were observed close to the catheter tip in most animals, whilst neither a clear correlation could be drawn if or to which extent these lesions had influenced blood pressure data nor how they developed over time. Radiotelemetry offers continuous long-term blood pressure measurement in conscious freely moving mice and thus clearly outpaces other techniques analyzing blood pressure. However, this study suggests that pressure recordings should be interpreted in association with the results of a histological assessment, especially if unexpected changes have been observed during blood pressure measurement.

PA30 Effects of Red Wine Polyphenols on bone mechanical properties in ovariectomized rats.

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Phytoestrogens have been suggested as an alternative treatment for postmenopausal osteoporosis (1,2). This study investigated the effect of Red Wine Polyphenols (RWPs) extract, with proven in vitro mild estrogenic action, on the mechanical strength of ovariectomy-induced osteoporotic bone.
Materials and methods: Thirty-five female mature (10-month-old) Wistar rats randomly allocated into Ovariectomy (OVX) and Control group (CTRL). The OVX group was divided into 2 groups, an untreated group (OVX, n =11) and a group treated with RWPs extract (RWPs, n = 11). RWPs extract was administered through their drinking water (dose 50 mg/kg body weight/day), starting immediately after ovariectomy. After euthanasia at 6 months, the left femurs were isolated, cleaned of soft tissues and kept at -20°C to determine the bone mechanical properties, which were measured using the three-point bending test (3PB). One of the two fragments was potted in dental resin and polished so as to record the full cross-sectional shape. Simultaneously the exact deflection of the bone was calculated with the use of an optical extensometer (RTSS HR Video Extensiometer, Limess). All data from video were recorded through TestWorks programs 4 which controled the charging framework and RTSS Control & Analysis Software which controlled optical extensometer. The processing of the images was done with the stereoscope using the Solid Works software, so it became possible to calculate the Exact Bone Strength (σ). Results: 3PB revealed that the maximal load before fracture (Fmax) was 110.80 N for the CTRL, 103.97 N for the OVX, and 112.18 N for the RWPs group. Mean values of Energy were 14.67 mJ (CTRL), 16.46 mJ (OVX) and 16.37 mJ (RWPs) respectively, without significant differences between groups. The mean values of σ were 175.2 N/mm2 for the CTRL, 155.2 N/mm2 for the OVX and 198.01 N/mm2 for the RWPs group. σ was significantly greater in the RWPs group as compared with OVX group (p=0.03). Additionally, σ was significantly lower in the OVX group as compared with the CTRL group (p=0.022). Conclusions: RWPs produced a remarkable effect on bone strength in the ovariectomized rat model. This is the most important variable determined as it directly expresses the resistance of the whole bone to fracture, incorporating both its elastic and plastic behavior.

PA31 Investigation of the effect of Chios mastic gum on ovariectomy-induced bone loss in rats

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Several plant extracts are consumed by humans as alternatives to pharmaceutical treatments for their beneficial effects on bone, some of which are attributed to their antioxidant action. Chios mastic gum (CMG), in addition to its antioxidant effects, has ingredients belonging to the triterpenoids (1), which have a significant protective effect on bone mineral density (BMD) (2). This study aimed to investigate CMG's potential beneficial effect on the ovariectomized rat model of osteoporosis.
Materials and methodsTwenty mature (9-month-old) intact female Wistar rats were randomized into two groups. The first group was ovariectomized (OVX, n=10) and the second group (OVX+CMG, n=10) was ovariectomized and administered Chios mastic gum per os post-operatively at a dose of 0.83 g/Kg per day. BMD measurements were carried out in vivo under anaesthesia at baseline, three and six months after ovariectomy. Results BMD percentage change from baseline (mean ± SD %) of the total tibia was evaluated for each group. At 3 months of treatment, there was a statistically significant difference between OVX+CMG group (-5.72 ± 3.57) and OVX group (-12.02 ± 4.19) p=0.003. At 6 months, their statistically significant difference was greater (OVX+CMG: -8.40 ± 4.83, OVX: -18.15 ± 3.82, p less than 0.0005). BMD percentage change from baseline to 3 months of the proximal tibia was statistically significant higher in OVX+CMG group (-8.13 ± 4.94) compared with OVX group (-28.21 ± 8.43) p less than 0.0005. At 6 months, the statistical significance between groups was similar (OVX+CMG: -21.52 ± 3.99 vs OVX: -36.73 ± 5.95, p less than 0.0005). Discussion and conclusions Our results indicate that the administration of Chios mastic gum had a significant protective effect on the BMD of the ovariectomized rat model of osteoporosis, both at three and six months.

PA32 Cryopreservation of mouse 2-cell embryos at IGC: in vitro fertilization efficiency rates in different genetic backgrounds

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The cryopreservation service of the Instituto Gulbenkian de Ciência (IGC) in Oeiras, Portugal, has contributed for a [2]-cell embryo biobank of around [130] mouse lines mostly produced by in vitro fertilization. Our work was firstly based on Naomi Nakagata's protocol, a method optimized in C[57]BL/[6] genetic background. By using the FERTIUP & CARD Medium of Cosmo Bio, we found a significant improvement in the fertilization efficiency in all genetic backgrounds tested, with a higher prevalence on FVB/N.

The rapid evolution of biomedical research and molecular genetics leads to an increase number and variety of new mouse strains. When no longer required, cryopreservation of mouse germplasm greatly reduces maintenance costs of live animals, freeing space for new lines. Also, it safeguards valuable lines against infectious outbreaks or natural disasters. The Mouse Facility of the IGC provides to investigators the cryopreservation service of mouse 2-cell embryos and sperm. Here we report the results obtained in the last 3 years, when we started the cryopreservation of around 130 mouse lines mostly by in vitro fertilization (IVF). We initially followed the protocol published by the European Consortium Infrafrontier/EMMA for freshly harvested sperm using TYH + methyl-β cyclodextrin as sperm pre-incubation medium in addition of reduced glutathione (GSH) supplemented fertilization medium. This protocol is based on methods developed by Naomi Nakagata's laboratory, at Kumamoto University and was optimized in C57BL/6 background. With this method we registered fertilization efficiency rates of 24% in C57BL/6 mouse strains, 28% in FVB and

22% in Balb/c. In 2014 we have started using the FERTIUP & CARD Medium from Cosmo Bio. We report here a relevant increase in the fertilization efficiency with this recent method. Analyzing the results per genetic background we could observe that not only C57BL/6 mouse strains (48%) but also FVB (72%) and Balb/c (40%) backgrounds showed noticeable improvements. All the results described here summarize all IVF sessions done, including sessions with 0% of efficiencies. The final step of our protocol is ensuring the quality and viability of the frozen material. For this, a straw of 2-cell embryos from each embryo freezing session is thawed and embryos are cultured in KSOM media to develop into blastocyst stage in vitro. Another Quality Control procedure we have implemented in our Facility is embryo revitalization, which consists in thawing a straw of 2-cell embryos followed by surgical embryo transfer to pseudo-pregnant recipients. This is routinely done for every strain with at least 200 embryos cryopreserved. Upon a viable progeny with the expected genotype, the strain is considered secure and the live animal resource is terminated. By constantly updating our procedure, we were able during the last 3 years to create an arquive of 70 secured lines, having at the moment around 60 more lines in the cryopreservation process.

PA33 Generation of a murine transgenic model for in vivo endothelial-specific and conditional expression of human apolipoprotein E3

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We aimed to generate a transgenic model to induce the conditional expression of hapoE3 specifically in the vascular endothelium using transcriptional transactivation dependent of tetracycline.
Materials and methods. Molecular cloning techniques were used to obtain two transgenes: (i) Tek/Tie2promoter-Tet3G-Tek/Tie2enhancer (Tek Tet3G, 5200pb) containing transactivator protein Tet-On3G gene under control of endothelial specific promoter Tek/Tie2 and the specific endothelial enhancer Tek/Tie2; and (ii) TRE-hapoE3 (5523pb) containing the hapoE3 gene under control of PTRE3G inducible promoter. BPAEC, RAW264.7 and HTB-11 cells were transfected with plasmids containing either the endothelial Tek/Tie2 promoter or the promoter and the endothelial Tek/Tie2 enhancer to test their functionality and specificity by measuring their ability to activate and enhance the luciferase gene transcription. To test the ability to induce conditional expression of hapoE3, BAEC, PBAEC and HTB-11 cells were co-transfected with plasmids containing the transgenes, followed by cultivation in medium with or without Dox and Western blot analysis. Transgenesis experiments were done by pronuclear injection in fertilized eggs obtained from FVB female mice. Animals positive for the transgenes, were identified by PCR genotyping the offspring from the first generation. Results. Results showed that the endothelial promoter Tek/Tie2 is activated in BPAEC, but not in Raw 264.7 or HTB-11 cells. The endothelial enhancer Tek/Tie2 increased the activity of Tek/Tie2 promoter (≈ 1.8 fold) specifically in BPAEC and had no effect on its activity in Raw 264.7 or HTB-11 cells. BAEC and BPAEC co-transfected with both pGL3-Tek/Tie2promoter-Tet3G-Tek/Tie2enhancer and pTRE3G-hapoE3 plasmids expressed hapoE3 only in presence of Dox in culture medium, while HTB-11 cells did not express hapoE3. Following transgenesis, we obtained one founder for the TRE-hapoE3 line and four founders for the Tek Tet3G line.Discussion and conclusions. The founder animals were used to develop a double transgenic mouse in which the expression of hapoE3 could be induced by Dox administration. To ensure its expression in absence of endogenous apoE, the double transgenic mouse were bred on the apoE deficient mouse. Acknowledgements. Work supported by PN-II-ID-PCE-2011-3-0591 197/2011 and the Romanian Academy. The author acknowledges the support of the strategic grant POSDRU/159/1.5/S/133391-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007 – 2013.

PA34 Access to shelter in metabolic cages can result in less body weight loss for mice

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The use of metabolic cages is common in different types of research, and it is considered a stressor. An attempt to reduce this stress by giving mice access to shelter in the metabolic cages is described.
Introduction An elevated level of albumin in the urine is a result of leakage across the kidney barrier and the use of 24h urine collection to calculate albumin leakage into the urine is an established measurement of kidney injury in rodent models. The time spent in the metabolic cages for urine collection is stressful for the animals. The animals are single housed on grid flooring and without bedding in the metabolic cage. Weight loss, lowering of the body temperature and ruffled fur coat can be seen in mice after metabolic caging. Prolonged stress is generally accepted to affect factors like kidney function and pharmacokinetics, so reducing stress in caged animals would likely result in more reliable research data. Also, Kalliokoski et al (2013) has concluded that mice do not habituate to metabolic cages, so making an effort to refine the use of metabolic cages should be done. Description In an attempt to reduce the stress, a small plastic igloo shelter was placed in the metabolic cages close to the water bottle. Mice can seek shelter in the house, thus reducing the stress and heat loss from single housing on grid floors. The rounded shape of the igloos prevents the mice from climbing the shelter and urine and faeces from accumulating on the roof. The mice were treated with streptozotocin in order to develop diabetes. Results Different strains of mice were used, both DBA and 129sv. When using DBA mice, the mice lost on average 4.3% of their body weight in the week after caging, without the shelter. In the study using shelters the average weight loss was 1.4%. When using 129sv mice no differences were seen in body weight or in excretion of corticosterone in either faeces or urine. Conclusions Mice of both strains used the igloos at all times. The use of shelters reduced weight loss in DBA mice during metabolic caging, but no weight loss were seen in 129sv mice regardless of shelter. Different mouse strains react differently to stressors, and some are more prone to stress than others, which can explain the different result from different strains of mice. The effects seen are likely due to reduced stress and reduced loss of body heat in mice allowed to seek shelter while single housed in metabolic cages. Use of the igloo did not compromise urine collection. Even though we cannot prove the benefit of shelters for all strains of mice, we still believe that the fact that the mice prefer to stay inside the igloos is reason enough for us to keep using them.

PA35 Can we use HbA1c as a diagnostic tool in mouse models of diabetes?

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Glycated haemoglobin (HbA1c) is the best indicator for long term glucose concentration. In mice, there are no recommended devices or standards for HbA1c. Glucose metabolism characterisation is mainly based on fasting glucose, glucose and insulin tolerance tests (GTT, ITT). With 1µL blood required, HbA1c point-of-care (POC) analysers are available, allowing improve refinement. Using one POC device (DCA-Vantage, Siemens), our aim was to establish a cutoff for HbA1c in diabetic & non-diabetic mice.
Methods Data from previous experiments with diet-induced (60% fat content) diabetic C57Bl/6J, & normoglycaemic B6;129S4-Pcmt1tm1Scl mice were used. Body weight (BW), fasting glucose (Glucocard-G meter), HbA1c, OGTT & ITT were assessed. Diabetes was defined as OGTT glucose concentrations >200 mg/dL. Correlations with HbA1c (linear regression; Pearson's test) and COR-curves were evaluated to establish a cutoff equivalent to a glucose concentration of 200 mg/dl, & the specificity/sensitivity for diagnosis. ResultsA total of 225 HbA1c measurements from 82 C57Bl/6J & 24 B6;129S4-Pcmt1tm1Scl mice were available. HbA1c (4.6% [3.4-7.3]) correlated positively with fasting glucose, OGTT peak, AUC and ITT glucose nadir (p200 mg/dL, n=173 HbA1c), AUC, BW, glucose nadir & fasting glucose were the parameters most strongly correlated with HbA1c & the diagnosis of the disease (p<0.001). Median HbA1c for diabetes was 4.5% [3.4-7.3] & for non-diabetes 4.10% [3.4-4.7]. COR-curves showed an optimal cutoff for the diagnosis of diabetes at 4.45% (specificity 64%; sensitivity 60%).Based on the present results, HbA1c seems to be a suitable diagnostic tool for diabetes in mice and a cutoff of 4.45% is proposed for the diagnosis of diabetes in the diet-induced diabetic C57Bl/6J. Indeed, standardised, internationally accepted, diagnostic criteria are still to be established.

PA36 CpG-induced specific immunotherapy to the major cat allergen Feld 1. Analysis in a murine model of asthma

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Recently, we have developed an effective CpG-based specific immunotherapy (SIT) to the major cat allergen Feld 1 in a murine model of allergic airway inflammation. The shift towards a tolerizing reaction was observed at the levels of airway hyperresponsiveness (AHR), lung inflammation, secretion of cytokines and humoral response (IgE). This work aims to clarify the mechanism of regulation by which the Feld 1-CpG based SIT exerts its tolerizing effect.
Materials and Methods: BALB/c mice were sensitized 3 times with Feld 1 + Al(OH) 3 by intraperitoneal (IP) injections (Days 0, 14, 28), generating a systemic Th2-type response to the cat allergen. For SIT, animals were 3 times IP-injected with Feld 1 + CpG (days 42, 56, 70). Subsequently, under isoflurane anesthesia, mice were challenged intranasally (NI) with Feld 1 on days 82, 83, 84 to induce asthma. In order to test for the specificity of the reaction, another series of mice were primed with 3 IP of Feld 1 + Al(OH) 3 and received 3 additional IP of PBS-CpG. These mice were also challenged with 3 NI with Feld 1 (control mice). At day 85, mice were anesthetized (IP injection), tracheotomized and mechanically ventilated to evaluate their airway hyperresponsiveness by measuring lung resistance and dynamic compliance in response to increasing concentrations of inhaled methacholine (with FlexiVent device). After FlexiVent measures, broncho-alveolar lavage fluids (BALFs) were recovered by washing the lungs with PBS 5% BSA followed by rinsing with PBS. Different cells types were studied and cytokines were quantified. In parallel, Ig profile were analyzed in the sera of the different mice. Results: Feld 1-CpG treated mice showed a reduction of pulmonary resistance, a significantly decrease in eosinophils infiltration in the BALFs, combined to a reduction of Th2 cytokines secretion but with no increase in TGF-beta and IL-10. These immunomodulations were also accompanied by the secretion of TNF-alpha in the BALFs. The BALF analysis of PBS-CpG treated mice showed the same but less pronounced effects, with the exception that TNF-alpha was not detected. The reduction of specific IgE in the serum of Feld 1-CpG treated mice was accompanied by a highly significant increase in specific IgA and IgG1, not seen in the PBS-CpG treated mice. Discussion and conclusions: These findings provide evidence that the immunotherapeutic effects of CpG in this murine model of Feld 1-SIT rely on both allergen-specific and -nonspecific driven mechanisms. We suggest TNF-alpha to play a role in the success of the immunotherapy since it has been reported that TNF-alpha may activate regulatory T cells via the TNFR2 receptor. We intend to develop the model in mice deficient for specific pathways to study the mechanisms of regulation engaged.

PA37 Thermography ear skin temperature in a neuro-ischemia rabbit model

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Evaluation of ischemia is important in animal models of wound healing in ischemic or neuro-ischemic conditions. This evaluation is important to control the good induction of ischemia and also to monitor ischemia evolution during the time course of a project. In this study, we show that the use of FLIR cameras is of interest in refining ischemia models in wound healing.
Ischemia and neuro-ischemia animal models are important in the evaluation of efficacy of new chemical entities or new medical devices targeting ischemic tissues in diabetes complications for example. New Zealand White rabbits (NZW) are widely used models in which ischemic or neuro-ischemic conditions can be induced in ear skin to evaluate wound healing principally. In these models, evaluation of skin surface temperature as a marker of ischemia, is usually done using surface temperature probes or using Laser Doppler Imaging (LDI). Surface temperature probes are easy to use and affordable, however, thermal mapping of ear skin using LDI is expensive and can be time-consuming. In this work, we have evaluated skin surface temperature from ears of neuro-ischemic rabbits using low cost and affordable infra red thermal camera (FLIR). Briefly, neuro-ischemia was surgically induced on left ear of 6 NZW by ligating central and rostral arteries, central and rostral nerves while caudal artery and all veins were left untouched. Right ear of each rabbit was not induced for ischemia and was used as control. Thermal imaging was performed every 2 days for 3 weeks and was compared to surface temperature measurement using infra-red commercial surface thermometer. Results show that thermal camera acquisition is easy to set up and results are fast to obtain with temperature visual mapping. Correlation with surface temperature probes was excellent. Thermal imaging using FLIR camera can help to refine ischemia models and should be generalized to other ischemic models.

PA38 Dose-dependent effects of Amphimas pterocarpoides plant extract on bone mineral density and strength in estrogen-deficient ovariectomized Wistar rats.

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Hormone replacement therapy (HRT) is crucial in osteoporosis management but prolonged use has been associated with various adverse effects, which has led to an increased demand for alternative therapies such as dietary natural plant estrogens. In the present study, we assessed the phytoestrogenic potential of Amphimas pterocarpoides (AP) extract, extensively used in Cameroon traditional medicine for treating a variety of hormone related disorders, on the ovariectomy-induced bone loss in rats.
Materials and Methods: Fifty-five female 10-month-old Wistar rats were used. Ovariectomy (OVX) was performed in 44 rats, while 11 rats were the Control group (CTRL). OVX animals were separated in the OVX group (n=13) and received drinking water ad libitum right after surgery until the end of the experiment, while the treated groups received AP extract at 50 mg/kg (ALow group, n=15) and 150 mg/kg (AHigh group, n=16). Measurements of Bone Mineral Density (BMD) of the proximal tibia and performed at baseline, 3 and 6 months after OVX. For the evaluation of trabecular bone BMD (TbBMD), a square ROI of 2 x 2 mm2 was selected in the proximal tibial metaphysis, 3 mm distal to the tibial plateau. After 6 months treatment, the rats were euthanized and femurs were extracted, on which biomechanical properties were evaluated. Results: The absolute values (mean ± SD) of TbBMD in the proximal tibial metaphysis in the OVX group were significantly lower than those in the CTRL at 3 months (0.258±0.031 g/cm2 vs. 0.351±0.018 g/cm2, p<0.001 ANOVA) and 6 months respectively (0.224±0.024 g/cm2 vs. 0.357±0.017 g/cm2, p<0.001). On the other hand, ALow group significantly prevented the decrease of TbBMD at 3 months (0.301 ± 0.011 g/cm2 vs. OVX 0.258±0.031 g/cm2, p<0.001) and at 6 months (0.267±0.017 g/cm2 vs. OVX 0.224±0.024 g/cm2, p<0.001). While, the absolute values of TbBMD in the AHigh group were significantly higher than those in the OVX group at 3 months (0.327±0.017 g/cm2 vs. 0.258±0.031 g/cm2 p<0.001) and 6 months respectively (0.314±0.018 g/cm2 vs. 0.224±0.024 g/cm2, p<0.001). The percentage changes of the mean values of the proximal tibia BMD from baseline to 3 months for CTRL, OVX, ALow and AHigh, were 6.254%, -21.822%, -14.512% and -4.421% while the percentage changes from baseline to 6 months were 8.108%, -32.134%, -23.985% and -8.144 respectively. The Fmax and the Stress applied, were significantly higher in the ALow group compare to the OVX group (Fmax 112.69 Mpa, Stress 180.5 Mpa Alow group vs. Fmax 96.9 Mpa, Stress 158.48 Mpa OVX group, p<0.001 and p<0.003 respectively). AHigh group presented higher values in Fmax and Stress in comparison to the OVX group, but without statistical significance. Conclusion: Administered AP extract for 6 months is effective in improving bone quantity and quality, surrogate markers of reduced risk of fracture.
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PA39 Study of the bone density of the total and proximal tibia in rats which have been born with intrauterine growth restriction.

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Intrauterine Growth Restriction (IUGR) has been associated with increased prenatal and postnatal morbidity and mortality, as well as a variety of maternal-fetal pathological conditions. Furthermore, infants may fail to reach their expected growth potential. The aim of the present study was to investigate the effect of intrauterine growth restriction on bone density of the total and proximal tibia of growing rats.
Materials and methods: Six first-time pregnant Wistar rats were obtained at 13 days of gestation; 3 of the mothers underwent a model of 50% food restriction until the end of pregnancy (21 days) and 3 of the mothers were fed ad libitum. The pups were culled to 8 (4 males and 4 females) per litter to normalize rearing. The offspring groups of investigation were: Group A: 12 male rats born IUGR and Group B: 12 Control male rats. Both groups were postnatally fed ad libitum. The experiment ended when the male rats reached 5 months of age. Results: Between the 2 groups there was no statistically significant difference in the litter size. IUGR newborn pups presented lower birth weight <2SD. Dual energy X-ray absorptiometry was performed at 2 and 5 months. Two regions of interest were placed, the first comprising the whole tibia while the second in the proximal tibial metaphysis. All values are presented as mean±SD mg/cm2. Two-way ANOVA model revealed statistical significant differences between groups for both total and proximal tibia at 2 and 5 months respectively. For total tibia at 2 months of age bone mineral density (BMD) was 0.186±0.005 for the Control group while for the IUGR group it was 0.171±0.008 revealing statistically significant difference (p<0.005). At 5 months of age the respective values were 0.274±0.007 for Control vs 0.252±0.015 for IUGR (p<0.005). For the proximal tibia at 2 months of age BMD was 0.266±0.0029 for Control vs 0.237±0.022 for IUGR (p=0.017) while at 5 months the respective values were 0.404±0.021 Control vs 0.360±0.018 for IUGR group (p<0.005). In conclusion, the prenatally normally fed Wistar rats presented significantly higher values of bone density compared to Wistar rats that underwent food restriction prenatally, during the observation period.

PA40 Micro-sampling and micro-dosing in mini-rodents

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The pharmaceutical industry currently faces an increasing number of requests for nonclinical juvenile animal toxicity studies from the international government medicines agencies in support of pediatric drug development. These complex studies entail many restrictions related to the small size of the immature test system, typically the rat, which requires elegant and ethically acceptable solutions for test article administration and obtaining data on toxicity and toxicokinetic parameters.
With the increasing need for juvenile animal toxicity studies in support of pediatric drug development comes increasing complexity in study design and animal handling. Depending on the clinical program, treatment duration and development of organs of pharmacological or toxicological interest, each juvenile animal toxicity study needs to be specifically designed, using animals of a developmentally representative age correlating to the intended pediatric patient population (i.e. using pre-weaned rats when there is a clinical need for drug administration to children during the first year of life). These studies entail many restrictions related to the small size of the immature test system and its need for nursing and continuous maternal care within the litter. It becomes even more challenging when it concerns pre-term neonatal patients, requiring a juvenile rat study starting at the representative age of less than post-natal day (PND) 8. This starting age should only be considered if absolutely required for the pediatric drug development and it requires elegant and ethically acceptable solutions for test article administration and obtaining data on toxicity and toxicokinetic parameters as routinely assessed in adult general toxicity studies. In this regard, we developed in-house juvenile rodent expertise to facilitate the successful application of oral gavage dosing of newborn PND 1 rat pups. Dosing the fragile PND 1 rat pups was found to be feasible using appropriate sized flexible soft plastic gavage (feeding) tubes with a bulb shaped tip although still challenging in terms of avoiding pre-term pup mortality. Furthermore, implementation of capillary microsampling (32 to 60µl) for toxicokinetic purposes from as early as PND 8 pups via the tail vein was successfully applied and eliminated the need for terminal bleeds from satellite animals, which is more cost-effective (i.e. man-hours, resources and test article required), but moreover significantly contributes to the 3R principles as it resulted in a > 50% reduction (i.e. > 200 animals) in experimental animal use in just one single experiment. In addition, more detailed age-related exposure profiles can be obtained, assisting better assessment and prediction of the often observed ADME differences between juvenile and adult animals.

PA41 New-born rodents: a new method of euthanasia.

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One of the sensitive points of the animal welfare management is involved in the killing. To this end, the Directive 2010/63EU gives the general rules of the conditions of killing and applicable methods. Some methods may be problematic for intensive use at certain stages of development, particularly during the postnatal phase. This is the case of the methods recommended for rodent newborns; cervical dislocation or decapitation. Both techniques of euthanasia for the postnatal phase have major defects in their realization; the visual result and persistence of post-mortem reflex movements. These elements are likely to offend the people in charge of this task especially if the number of animals to euthanize is very high as for a breeder. We must put to death over 5000 newborns a week and the methods recommended by the new directive aren't suitable for a large number of euthanasia. Indeed, the ethical criteria for animal experimentation killing are: 1) for the animal, the killing must be non-anxiety, painless and must produce a loss of consciousness instant and very quick death; 2) for the performer, the method must be safe and not cause an emotional shock. Thus, the goal for JANVIER LABS was to find an alternative method to the decapitation or cervical dislocation for rodent newborns which respond to the requirements of the directive but also to the moral protection and welfare of operators in charge of this act. The search for an alternative method to decapitation or cervical dislocation was based on the inability of newborn rodents less than 7 days to maintain their body temperature, the effects of cold and hypothermia phenomenon. Indeed, in rodents, the newborn has an efficient thermoregulation on the 7th day with the appearance of a transitional duvet and the perception of the environment appears essentially touch on the first 7 days of age. The trail of a deep and rapid hypothermia as killing system meets the requirements of the regulation and welfare of staff in charge of its implementation of its anesthetic effects, its protection of the central nervous system, rapid death by cardiac arrest. Hypothermia is caused by a decrease in temperature of the animal's environment using the heat loss to the maximum by a convection effect. In practice, the process has been validated by placing pups of less than 7 days in cages of euthanasia. The cages are then placed in a freezing cell to have a descent of the temperature by a continuous injection of nitrogen into the atmosphere of the cell and a continuous discharge of the injected gas (no initiation of hypoxia). A 4-minute cycle with a temperature down to 100 ± 4°C allows a death of all newborns less than 7 days. The benefit of this method is to have a short cycle for killing, an anesthetic effect and confirmation of death by complete immobility of animals and also the moral protection and welfare of the operators in charge of this act.

PA42 Rat brain volumetric differences related to domestication. In vivo 7T MRI study in wild rats vs. albino and pigmented laboratory rats

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Objective: Domestication of the Norway rat for laboratory purposes began at the mid-nineteenth century. Since then, selective breeding and chronic exposure to artificial environment resulted in morphological and biochemical changes in the nervous system. Certain ocular pathologies developed, mostly in albino strains. Also, wild and laboratory rats display markedly different behavior. Wild ones are more aggressive, skittish and neophobic.
Aim: We hypothesized that the aforementioned differences are correlated with differences in brain morphology, in particular in structures related to processing of visual information. The aim of the present study was to compare volumes of vision-related brain structures in wild and in domesticated albino or pigmented rats. Methods: Measurements were carried out on adult male (9-10 week-old) rats sourcxed from 3 populations: (1) laboratory-kept Warsaw Wild Captive Pisula-Stryjek rats (WWCPS, n=9) as a model of wild rats, (2) strain of pigmented laboratory Brown Norway rats (BN, n=8) and strain of albino Wistar rats (WI, n=8). WWCPS were handled with custom-built devices and techniques for transporting animals

between cages, and separating and catching them during preparation for imaging. Animals were anesthetized with isoflurane (4% for induction and 1.5-2% for maintenance, supplied with oxygen). The rats were placed in a 7T small animal-dedicated magnetic resonance tomograph (BioSpec 70/30USR; Bruker BioSpin, Ettlingen, Germany). High resolution structural imaging with (TR/TEeff = 5000/30ms, RARE factor = 4, spatial resolution = 125µm x 125µm x 500µm, 54 slices, no gaps, number of averages = 3). For the volumetric analysis we employed Schwarz Rat Atlas published online with small animals SPM toolbox SAMIT (http://mic-umcg.github.io/samit/). Data were preprocessed with N4 intensity non-uniformity correction algorithm implemented in Slicer 3D (http://www.slicer.org/) and processed using SPM software (http://www.fil.ion.ucl. Ac.uk/spm/) and custom-made MATLAB scripts. Statistical analysis was performed with Kruskal-Wallis test followed by Dunn's multiple comparisons post hoc test. Results: Brain size was significantly (P<0.001) higher in Wistar rats than in WWCPS rats but not in BN rats. Relative volume of visual cortex was significantly greater in WWCP than Wistar rats (P<0.001) whereas difference between WWCP and BN was not significant. No significant differences were detected for volume of superior colliculi (main primary target of retinal ganglion cells projections in rodents). Conclusion: The present data show that domestication process in rats markedly affected brain morphology. In particular, albino rats display lower volume of visual cortex, possibly related to retinal degeneration and development of blindness.Study supported in part by Polish National Science Centre grant No. 2012/07/D/NZ4/04199.

PA43 MRI, PET/CT and histopathological examination in visualizing neoplastic lesions in rats with p53 gene knockout

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The Tumor Protein 53 (Tp53, p53) is the most studied tumor suppressor. p53 plays a role in cell cycle control, apoptosis, angiogenesis, carcinogenesis, senescence, DNA repair, and changes in metabolism. Homozygous and heterozygous p53 TGEM®Rats develop a wide variety of malignant tumors, including sarcomas and lymphomas. Homozygous p53 TGEM® Rats develop tumors at 3-4 months
Materials and methodsIn this study we used p53 knockout males rats (aged 3,5 - 4,5 months). Examination was performed using a magnetic resonance imaging scanner Bruker 7T, using a signal acquisition set consisting of a transmitting volume coil (dia. 86 mm) and a superficial receiver coil, placed in the area a region of interest. Structural imaging was performed using the sequence: TurboRARE-T2 2D (TR = 2500ms, TEeff = 36ms, Rare Factor = 8, NA = 2, spatial resolution = 0,156mm x 0,156mm x 0,8mm) diagnosing the presence of cancerous tumors. We also used Albir PET / SPECT / CT Preclinical Imaging System which uses isotopes of fluorine (18F). ResultsIn selected 2 rats performed a detailed anatomopathology study. The tissue samples were taken from tumors and internal organs for further histopathological examination. The histopathological examination in rat No. 1 were found hemangiomas sarcomas (haemangiosarcoma), wherein the infiltrative tumor of the thoracic muscles, showed a very aggressive growth with solid foci of undifferentiated sarcoma. Additionally was visible stimulation of the rat immune system. In the mesenteric lymph node found a cluster of cells with the morphology of sarcoma. Rat No. 2 had tumor which was low-differentiated and highly malignant (carcinoma), while, in the adrenal gland, in the zona fasciculata found cluster of atypical cells.Discussion and conclusionsSummarizing - study using magnetic resonance is a sensitive method for imaging neoplastic lesions in rats, but observed changes require testing by other methods such as histopathology, because the image obtained in resonance does not allow for clear identification of the type of tumor or the presence of micrometastases or forming small, atypical cell clusters. Due to the high degree of malice and low differentiation, tumors in p53 knockout rats require an analysis with immunohistochemical methods

PA44 Anterograde axonal transport impairment precedes intraocular pressure elevation in DBA/2J mouse model of hereditary glaucoma

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Purpose: Axonal transport impairment is an important hallmark of neurodegenerative diseases including glaucoma and could be a key factor in developing glaucoma-related neurodegeneration outside retina (extra-retinal neurodegeneration). However, it remains elusive when these deficits develop and whether they can precede intraocular pressure (IOP) elevation
Eim:In the present study we used a quantitative manganese enhanced magnetic resonance imaging (MEMRI) to investigate early and delayed age-dependent axonal transport alterations in a model of spontaneous glaucoma-like pathology (DBA/2J mice). Material and methods: 3-, 6- and 14-months old DBA/2J mice and age-matched control animals (C57Bl/6)underwent IOP measurements in both eyes and were subsequently subjected to unilateral intravitreous administration of MR-visible axonal tracer manganese chloride to track fast axonal transport along axons of retinal ganglion cells. 24 hours after the injections animals were subjected to T1-weighted imaging and T1 mapping, T1 relaxation times for regions of interests (ROIs) centered on vision-related brain structures (superior colliculus, SC and lateral geniculate nucleus, LGN) were calculated. Measurements on set of phantoms served to determine relaxivity of manganese ions in our setting and allowed estimation of local manganese concentration in SC and LGN. Results: IOP was significantly higher in 6-months old DBA/2J mice than in age-matched controls (18.4±1.0 mm Hg vs. 13.6±0.3 mm Hg, P<0.001) but not in 3 months old animals. Intravitreous manganese administration resulted in significant enhancement within SC and LGN in all groups of C57Bl/6 animals and allowed in vivo quantitative evaluation of anterograde axonal transport by estimation of local, manganese concentration in SC and LGN after the injection. In 3-months old C57Bl/6 mice local manganese concentration in ROI centered on SC was estimated to be 23.20 ± 1.59 µmol/l and decreased with age. In 14 months old DBA/2J mice the enhancement was dramatically lower than in age-matched controls (<1 µmol/l both in SC and LGN). Moreover, in 3-months old DBA/2J local manganese concentration in SC was already lower than in C57Bl/6 mice and corresponded to values in 14-months old controls. Conclusion: Anterograde axonal transport was severely impaired in DBA/2J mouse model of glaucoma. The impairment was very pronounced in animals with advanced glaucoma-like pathology but mild axonal transport impairment was an early event in the pathology and preceded IOP elevation.Such early changes in axonal transport could be a target for neuroprotective treatment strategies. This study was supported by Polish National Science Centre grant (2012/07/D/NZ4/04199) to MF.

PA45 Investigation of conception on expression of integrin αvβ3 and VEGF in Wistar rat with endometriosis

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Allografts uterus were transplanted into 38 female Wistar rats to establish endometriosis model, Histological analysis was used by transmission electron microscope, and the expression of integrin $\alpha v\beta 3$ receptor and VEGF gene in endometrial was tested by RT-PCR method; in the meantime, the contents of estrogen and progesterone in serum was detect by elisa technique. All this are to find how conception can affect the expression of integrin $\alpha v\beta 3$ and VEGF in Wistar rat with endometriosis.
Objective: To investigate the expression of integrin $\alpha v\beta 3$ and VEGF gene in endometriosis model rats 4.5 days after mate and to detect the content of estrogen and progesterone, which can explains its relationship with infertility. Methods: Allografts uterus were transplanted into 38 female Wistar rats to establish the uterine rectal pouch endometriosis model. Male rats were permitted to approach the female models 8 weeks after transplantation, The eutopic and ectopic endometrium were collected 4.5 days after mate. Histological analysis was used by transmission electron microscope, and the expression of integrin $\alpha v\beta 3$ receptor and VEGF gene in endometrial was tested by RT-PCR method; in the meantime, the contents of estrogen and progesterone in serum was detect by elisa technique. Results: The expressions of integrin $\alpha v\beta 3$ and VEGF in uterine rectal pouch endometriosis model rats are significantly lower than the abdominal wall endometriosis model rats ($p<0.05$). And conception can both improve the expression of the two gene but still can't return to the normal expression level (p0.05);The content of estrogen in uterine rectal pouch endometriosis model rats are significantly lower than the normal level ($p<0.05$), but the content of progesterone are significantly higher than the normal level ($p<0.01$). Conclusion: the expression of integrin $\alpha v\beta 3$ and VEGF gene were affected by endometriosis as well as the content of estrogen and progesterone. The expression level of this two gene and the content of estrogen and progesterone all can change the ability of endometrial to accept embryo, eventually it will affect the pregnancy rates.

PA46 Effect of polydatin on glucose and lipid metabolisms in experimental diabetic models through the Akt signaling pathway

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Polydatin has various biological effects on inflammation, oxidation, and cardiovascular protection. Recently, the lipid regulation of polydatin has gained considerable attention, and a previous study has demonstrated that polydatin has a hypoglycemic effect on experimental diabetic rats
Polydatin has various biological effects on inflammation, oxidation, and cardiovascular protection. Recently, the lipid regulation of polydatin has gained considerable attention, and a previous study has demonstrated that polydatin has a hypoglycemic effect on experimental diabetic rats. Thus, we used a rat model induced by high-fat and -sugar diet with low-dose streptozocin and an insulin resistant HepG2 cell model induced by palmitic acid (PA) to further clarify the role of polydatin on glycolipid metabolism in experimental diabetes models and to explore the possible mechanism. Western blot assay was used to detect the phosphorylation levels of Akt, glycogen synthase kinase-3β (GSK-3β), the protein levels of glucokinase (GCK), glucose-6-phosphatase (G6Pase), low-density lipoprotein receptor (LDLR), and sterol regulatory element-binding transcription factor-1c (SREBP-1c). SREBP-1c nuclear translocation was detected using a laser scanning confocal microscope. Lipid accumulation was detected through oil red O staining. Glucose uptake was measured by flow cytometry. Commercial kits were used to measure triglyceride, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, glycosylated hemoglobin, glycosylated serum protein, hepatic glycogen, and glucose consumption. Polydatin effectively improved the glycolipid metabolism of diabetic rats, significantly increased glucose uptake and glucose consumption, and decreased lipid accumulation in HepG2 cells. Polydatin markedly increased the phosphorylation levels of Akt and GSK-3β, decreased the protein levels of G6Pase and SREBP-1c, and increased GCK and LDLR in diabetic rat liver and PA-induced HepG2 cells. These results indicated that polydatin apparently regulate glycolipid metabolism in experimental diabetic models, the underlying mechanism was probably related to the Akt mediating pathway.

PA47 Spontaneous generalized lipodystrophy with growth retardation in mice

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A new phenotype was detected during inbreeding of mice from the C selecting line. The C line was derived from the outbred stock of four inbred strains: A/St, C57BL/6, BALB/cW and BN/aW. This line was maintained at Warsaw University of Life Sciences and selected over 130 generations for high body weight. In 2008, a few breeding pairs from the C line mice entered the facility of the Department of Genetics at The Cancer Center and Institute of Oncology, where an inbreeding program was begun.
Materials and methods Newborns were evaluated no later than 24 hour after parturition. Puppies were observed every day and all unusual signs were noted. To confirm genetic source of observed phenotype we have performed crossing potentially heterozygous male with female from AKR/W inbred strain. Genotyping was performed with 70 microsatellite markers. The linkage analysis was performed with MapManagerQTXb20. The whole exome sequencing was performed using Ion Proton platform. Results and discussion The new phenotype was manifested mainly by postnatal growth retardation, skin lesions on the feet, tail, and occasionally ear pinnae and eyelids, and subsequent motoric impairment. The first abnormal signs were visible from 2-4 days of birth – the affected animals were smaller than their littermates, they opened eyes about 2 days later than unaffected pups. During the 3rd week of life skin lesions became visible and at weaning parchment–like skin was observed. At weaning the body weight of mutated mice was about half that of the unaffected littermates. Motoric impairment was usually observed from 1-3 months of age. Animals presented first with an uncertain, swinging gait, followed by postural abnormalities and a sliding motion using the sides of the body during movement. Despite these severe symptoms no signs of pain were observed. Both sexes of the altered animals showed no signs of sexual instinct and were infertile. The affected mice usually died between 3 and 6 months of age (mean life time = 141 days). Necropsy revealed generalized lipodystrophy and atrophy of the thymus. The behavioral or anatomical changes we observed were not found in the parental strains or in the animals from the selecting line C (before the start of inbreeding). Pedigree analysis has shown that the observed changes are inherited as an autosomal recessive trait and was found in 47 of 206 mice, both males and females. Autosomal recessive transmission of the observed

phenotype was confirmed by crossing carriers from the C line with mice from AKR/W inbred strain. Genetic mapping determined the locus linked to the altered phenotype at the distal part of Chr4 (>70cM). Using exome sequencing three candidate mutations were identified at the critical region in the loci: Vps13d, Mtor and Pgd but none of them showed co-segregation with the observed phenotype. We suggest that the phenotype described above can be caused by a new mutation in the non-coding part of gene in the critical region of Chr4.

PA48 Reduction of animal usage by using cassette dosing approach and cross-over design for early pharmacokinetic (PK) screening in drug discovery phase

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PK studies of small molecule test compounds in rodents represent a cornerstone of contemporary drug discovery programs as these PK data are often used to rank-order compounds mainly based on oral bioavailability and blood clearance, to establish critical structure-activity relationships useful for guiding medicinal chemistry efforts. Herein we describe an animal- and resource-saving cassette approach associated with serial bleeding and cross-over design that allows high throughput in vivo PK screening.
Materials and methods: To maximize the animal usage, the male Sprague-Dawley rats (n=3 per group) with surgically implanted catheters for intravenous injection and blood collection or the mice (different strains and genders) were successively dosed with different compounds either single or in cocktails (cassettes, containing 4-7 new compounds plus a cassette reference standard) via intravenous (i.v.) and oral (p.o.) administrations. After each dosing, the blood (50 µL) is collected over 24 h and then analyzed by LC-MS/MS for compound concentrations. Results: The pharmacokinetic data, i.e. systemic clearance and oral bioavailability, obtained after single and cassette administrations in mice or rats were compared for more than 30 diverse compounds. Moreover, the reduction of the animal usage was clearly demonstrated by statistical evaluations. Discussion and Conclusions: In drug discovery using classical PK methods, large numbers of animals are needed, leading to high cost and slow turn over. The association of cassette dosing, cross-over design and serial method allow the use of few rodents for many compounds tested. In our group, only 3-6 mice were needed to assess the PK of 6 compounds and only 3 rats for 12 compounds. Furthermore, this approach allows comparing compounds with each other in a single animal and allows the scientist to distinguish inter-individual variation from other factors. Last but not least, the great alignment between the PK data obtained with cassette and conventional dosing suggest that cassette technology should be given consideration when planning early stage pharmacokinetic experiments.

PA49 Improving Receptor Autoradiography Images of rodent and human brain tissue (Replacement, Reduction and Refinement).

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Autoradiography (AR) is used in the preclinical evaluation of PET (Positron Emission Tomography) tracers. AR is a method to study the binding of PET tracers to slices of intact post mortem tissue (snap frozen). For the readout of the binding, phosphorimaging (PI) can be applied with a resolution up to 50 µm. [1] PI has the advantage of shorter exposure times. Preparation of the tissues, especially human brain tissue, is extremely important for optimal results. Being able to do reliable AR on post mortem human tissue reduces the use of animals. Here we present an optimised protocol for AR.
Methods For our optimised procedure we use frozen tissue slices of 10-20µm, sliced in a cryostat at -19 °C and thaw mount them on a superfrost plus glass slide, melting time at room temperature for 30 minutes. The tissues were dried overnight at 5 °C and stored at -20 °C until use. Just before the experiment, slices were washed with buffer and dried under a stream of air before use. For fragile tissues we used super frost plus gold glass slides and prolonged thaw mounting time, drying time and dried them with silica upon storage.Incubation with the PET tracer (P2X7 receptor ligand [11C]SMW139 or NMDA receptor ligand [18F]PK209) in a buffer solution at ambient temperature, resulting in the total binding. Incubation of the same with the addition of a high concentration (10 µM) of blocker (JNJ-47965567 for the P2X7 receptor, MK801 for the NMDA receptor) defines the non-specific binding. After incubation the slices were washed with buffer at 4 °C, dried and exposed to the phosphorimaging screen, which was read out after exposure using a phosphor imager (Typhoon FLA7000). To obtain maximal resolution, we compared first generation screens (Molecular Dynamics) to second (Amersham) and third generation phosphorimaging screens (Fuij). Results The new method we used provided reliable tissue preparation, some representative images are presented in Figure 1. Figure 1: AR images using our optimised protocol. Panel A.....Panel B..... First generation looks blurry compared to the 2nd and third generation screens. The 3rd generation images showed the highest resolution, see Figure 2 Figure 2: Comparison of phosphorimaging screens. Panel A: Molecular Dynamics, panel B: Amersham and panel C: Fuji. Conclusions Our improved method gives optimal AR results. Use of third generation Fuij screens generates high resolution images up to 25µm (40 pixels/mm). Acknowledgement This research has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F2-2011-278850 (INMiND).

PA50 Archiving and distributing Mouse Lines

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The Mary Lyon Centre, MRC Harwell, maintains a non-profit mouse archive that aims to collate a wealth of useful mouse mutants that will be publically available and accessible to the whole scientific community. We are able to offer this archiving service free of charge, with any costs recuperated from clients who order the lines.
Our team is highly skilled and proficient in the archiving of both sperm (5 mature males are required) and embryos (30 females and 5 males are required); with our dedicated quarantine unit allowing us to preserve germplasma from mice with very low health statuses. Recent advances in thermal controlled packages are enabling us to switch from live animal imports toward the transport of dissected epididymides. This tissue contains the germ plasma used for sperm freezing and completely removes the need to transport animals which is both an improvement in welfare

and costs. When a client orders a mouse line from the archive cryopreserved material is either directly shipped or is used to resurrect the stock. In the past the transport of frozen sperm and embryos required specialised equipment and the capability to handle liquid nitrogen. However alternatives now exist, enabling institutes to easily transport this type of material. Frozen sperm can be shipped in dry-ice parcels with no negative effect on the fertilisation rates. On receipt the client can either use the sperm directly in an IVF or it can be returned for long term storage. Frozen embryos can be thawed and shipped 4°C, ready for transfer at the receiving facilityTogether with the scientists who deposit at our archive we are looking to establish a cooperative future in mouse research that will benefit the scientists, funders and ultimately society as a whole through a better understanding of human disease.Further information on submitting and ordering mouse lines can be found at; https://www.infrafrontier.eu/ or if you would like further information please contact us at fesa@har. Mrc. Ac.uk.

PA51 Models & Resources for Type 2 Diabetes Research from The Jackson Laboratory

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Choosing the most appropriate model for diabetes research can be daunting. Many factors contribute to the complex disease presentation in humans and consequently no single mouse model can recreate all aspects of the human disease. However, several mouse models can be utilized individually or jointly to provide a platform from which aspects of the global Type 2 diabetes phenotype can be evaluated.
Comparisons between the most popular models for type 2 diabetes research, including the Lep ob/ob, Lepr db/db, and the C57BL/6J diet induced obesity (DIO) models, show that different genetic backgrounds and environmental conditions are able to model various stages of disease as well as different complications. Data comparing various metabolic parameters (clinical chemistries, glucose tolerance tests), energy balance and behaviors (body weight, body composition, thermoregulation, food consumption, etc.), and complications of diabetes (end-organ system assays) are presented in the context of short term as well as longitudinal model evaluation.

PA52 Murine model in the study of an endometrial chronic co-infection by Chlmaydia trachomatis and Ureaplasma urealyticum.

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C. trachomatis is considered a prevalence sexual transmission bacteria, it infects 90 million people annually and U. urealyticum shows a high affinity for epithelial surfaces,Due to the high incidence of human beings co-infection with these pathogens and because there is no model to investigate the pathogenesis of this mixed infection, our aim was to develop a murine model for the study of an endometrial chronic infection by C. trachomatis and U. urealyticum.
The pathogenic mechanisms of the genital tract infection by C. trachomatis murine variety (Chlamydia muridarum) are very similar to women genital tract in an acute infection. These similarities in the pathogenesis and the adaptive immune response generated after resolution of infection show the great usefulness of animal models to study the pathogenic mechanisms of this infection.Our aim was to develop a murine model for the study of an endometrial chronic infection by C. trachomatis and U. urealyticum. 60 mice C57BL/6Hsd (0044) 5 weeks aged were distributed in 5 groups (3 experimental and 2 control). Cytological and histological abnormalities were observed by diagnosis tests: direct immunofluoresce, API technology and IST2 selective culture. Cervical samples were analyzed with the chi-square test statistic Pearson ($p = 0.0001$). It was found that C. trachomatis and U. urealyticum induce significant cellular damage to the endometrial tissue. The cytological and histological alterations caused by C. trachomatis and U. urealyticum in murine model, are easily evaluated using tissue microarray because it allows the implementation of an optimized way of several cytohistological techniques. This murine model allows the study of the cytological and histopathological characteristics of the co-infection by C. trachomatis and U. urealyticumin in the genital tract. In agreement to the obtained results we corroborate that the infection caused by C. trachomatis and U. urealyticum induces cellular damage to the endometrial tissue.In the histological uterine analysis of groups 3, 4 and 5, the observed damage in the endometrial epithelium were: cellular mitosis accentuated, hyperplastic cells and areas of epithelial detachment of the basal membrane. In the endometrial stroma it was observed the presence of edematous tissue, besides polymorphonuclear cells, infiltratation and plasmacytes. Other observed characteristics were the presence of secretory impregnation and glandular cysts. Tissue alterations were accentuated in the 15th and 30th days, but diminished in the 45th and 60th days. The presence of Mycoplasma and Chlamydia were detected in the epithelium and endometrial stroma. The cytological examination of the vaginal discharge confirmed the presence of the inflammatory process in the groups 3, 4 and 5 (inoculated with C. trachomatis and U. urealyticum), characterized by epithelial cells, cellular debris and inflammatory cells, besides the presence of inoculated bacteria.

PA53 Toxicity evaluation of an alcoholic extract of epazote leaves (Chenopodium ambrosioides).

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C. ambrosioides is distributed throughout America. In Mexico as "epazote". In alternative medicine its oil is used as an anthelmintic. The aim of this study was to evaluate the effect of an extract of C. ambrosioides (2000 mg/Kg dose).10 female Long Evans rats, aged 28 days, into two groups. The first experiment, the extract was orally administered through a gastric tube. C. ambrosioides has no significant toxicity to the administered dose.
The existence of plants with high therapeutic potential is a pharmacological alternative of sturdy interest in the treatment of many diseases, hence the importance of conducting preclinical studies in order to detect possible toxic effects post administration. Nowadays, there is little information about the toxicity of plants used in alternative or traditional medicine. Such is the case of Chenopodium ambrosioides, which is widely

distributed in Mexico, it is commonly called as "epazote" or "paico" in South American countries. This is an aromatic perennial plant, with different uses throughout Latin America; its leaves, roots and inflorescences are used as a condiment or as an anthelmintic medicinal plant.In alternative medicine its oil is used as an anthelmintic for both human and animal. The aim of this study was to evaluate the effect of an extract of C. ambrosioides (2000 mg/Kg dose) in Long Evans rats to observe or discard signs of toxicity. The animals were kept in a temperature room of 22 ± 3°C and light/dark cycles 12/12 h. They were provided by commercial solid food and water for human consumption (both ad libitum). Biological models were preliminary observed and acclimated for 7 days before the experiment. For this study, 10 female Long Evans rats, 28 days old were used. They were separated into two groups. To the experimental one, the extract was orally administered through a gastric tube, prior fasting 16 to 18 hours. Five females in the experimental group were dosed administering a volume of 10 mL/Kg body mass of the test substance. The control group received 0.5 mL of saline solution. The experiment results show that there is no significant difference between control and experimental groups. The plant extract C. ambrosioides has no significant toxicity to the administered dose (suggested by the fixed dose procedure). We do not know if the active ingredient (ascaridole) of the plant could be affected by the collection site and life time, because its components vary according to their location and lifetime of the sample. All procedures were performed according to the Official Mexican Standard NOM-062-ZOO-1999 and 420 OECD Memorandum.

PA54 Patient-Derived Xenograft (PDX) Models of Glioblastoma: From Basic Research to Pertinent Preclinical Studies.

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Glioblastoma is the most malignant brain tumor with no effective treatments. Most clinical trials fail due to inappropriate selection of the compounds at the preclinical stage. Therefore, appropriate preclinical models are crucial for achieving better treatment outcome. Relevant models should reflect the clinical features of the disease, reproduce the genetic and histopathological heterogeneity of patient tumors and the complexity of drug distribution in the brain.
Materiel and Methods Patient-derived xenografts (PDX) are based on organotypic spheroids derived from mechanically-minced glioblastoma tumor biopsies, cultured shortly in non-adherent conditions. Spheroids are stereotactically implanted in the right frontal cortex of immunodeficient mice (NOD/Scid, nude). Upon tumor development, xenografts can be further used for the generation of organotypic spheroids and serial transplantation for several generations. During tumor growth xenografted mice are checked daily and pain is scored. Mice are sacrificed upon the first signs of pain or first neurological symptoms. All procedures are approved by the animal welfare structure of the Luxembourg Institute of Health and the national authorities responsible for animal experiments in Luxembourg (following the European Directive 2010/63/EU). Results Our PDX model of glioblastoma is characterized by a tumor take rate close to 100% and a very reproducible development time. For each given a tumor we observe three distinct histological tumor phenotypes: a highly "invasive", a highly "angiogenic" and an "intermediate" phenotype which combines invasion and vascular abnormalities. Typical glioblastoma characteristics such as pseudopalisading necrosis, invasion or microvascular proliferation are maintained. The PDX model maintains the patient-specific genetic aberrations through several generations. The model can be applied for analyses at different molecular levels including genomic, transcriptomic, proteomic and metabolomic assays. Using eGFP-expressing NOD/Scid mice allows to discriminate tumor and stromal cell populations and to characterize in depth intra-tumoral heterogeneity. Importantly the model can be reliably applied to study the efficacy of novel drug candidates and /or drug combination. Discussion and conclusion Glioblastoma PDXs based on organotypic spheroids represent a reliable and clinically-relevant animal model. It can be applied for accurate reproducible pre-clinical trials, including personalized medicine-based treatments. The use of this model should lead to a better evaluation of the efficacy of novel drugs, and increase the success of follow-up clinical studies. Although the use of immunodeficient animals limits potential applications for immune therapies, the role of microglia and macrophages – the main immune component of glioblastomas – can still be addressed in nude mice.

PA55 Case report of spontaneous lymphoma in patient-derived tumor xenografts: the importance of systematic analysis of xenografted human tumor tissue in oncology preclinical efficacy trials.

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Patient-derived tumor xenograft (PDX) model is now largely recognized as a powerful model for preclinical anticancer drug testing, as it recapitulates the intratumoral clonal heterogeneity, gene expression profile, key molecular alterations, histology and drug response of the patient tumors (1,2) Recently, large-scale in vivo screens ('PDX trials') are increasingly used to model inter-patient response heterogeneity and a 'one animal per model per treatment' approach is proposed (2).
In this context, we report here unintentional co-engraftment of lymphoma when passages for expansion of well-established human uveal melanoma xenografts. The MP42 uveal melanoma PDX model (passage 13th) has been subcutaneously engrafted from 2 Crl:NU(Ico)-Foxn1nu mice in 30 CB-17/Icr-Prkdcscid/Rj for PDX expansion with the aim of anticancer drug efficacy testing. Because of higher robustness and lower price, Crl:NU(Ico)-Foxn1nu are used for serial transplantation while CB-17/Icr-Prkdcscid/Rj are selected to promote metastasis development. As usually, the engrafted scid mice have been randomly divided into the different treatment groups when the tumors reached a volume of ~80 mm3. From 10 to 14 weeks after engraftment, 14 mice displayed cachexia, dehydration, weakness, whatever the treatment group, and have to be killed for ethical issues. At necropsy, pale tissue, large tumors at the engraftment site and splenomegaly were observed. Histological examination revealed a mixed tumor with both melanoma and lymphoma components at the subcutaneous site and lymphoma in the spleen. Death could be then attributed to metastatic lymphoma. All moribund mice had been engrafted with a tumor fragment from the same donor nude mouse. Two hypotheses could explain this lymphoproliferative disorder due to a common xenograft source: i) As EBV-associated human lymphomas have been reported in human solid tumor xenotransplantation in immunodeficient mice (3), a human origin can not be ignored. Nevertheless, these EBV lymphomas generally grew in the very early passages in immunodeficient mice while MP42 was used here at passage 13th; ii) A spontaneous lymphoma from the nude donor mouse is the most likely hypothesis and molecular analyses are required to confirm it. More importantly, this case report leads to the conclusion that every PDX tissue used for routine tumor passage and drug testing experiments needs to be carefully checked for its real nature, both at the beginning and at the end of the experiment. It is a key point to experimental validity, all the more when only 'one animal per model per treatment' is used in newly designed 'PDX trial'. This verification and the way it is done have to be clearly mentioned in Material and methods section but this is not the case today.

PA56 The requirement of nidogen 1-laminin γ1 interaction for Schwann cell differentiation.

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During peripheral nervous system (PNS) development, axonal sorting and myelination is accomplished by Schwann cells (SCs). Extracellular matrix components as well as basement membrane (BM) proteins such as laminins, and their receptors have been shown to be involved in these developmental events. Nidogen 1 and nidogen 2 are ubiquitous BM proteins with a high binding affinity to the laminin γ1 chain. We analyzed the role of both nidogen isoforms and its interaction with laminin in the murine PNS.
INTRODUCTION: During peripheral nervous system (PNS) development, axonal sorting and myelination is accomplished by Schwann cells (SCs). Extracellular matrix components as well as basement membrane (BM) proteins such as laminins, and their receptors have been shown to be involved in these developmental events. Nidogen 1 and nidogen 2 are ubiquitous BM proteins with a high binding affinity to the laminin γ1 chain. In the present study, we analyzed the role of the nidogen protein family and its interaction with laminin in the murine PNS. MATERIALS AND METHODS: We applied histology, immunohistochemistry, ultra-structural, morphometric, Western blot and proteome analysis for the investigation of sciatic nerves from nidogen 1-deficient (Nid1-/-), nidogen 2-deficient (Nid2-/-) and wild type mice at postnatal day 3, 7, 14, 21, 42 and 90. In addition, we analyzed a mouse strain with abolished nidogen-laminin γ1 interaction due to introducing a single-point mutation into the laminin γ1 chain in the germ line of mice (lamγ1N802S mutant mice). RESULTS: Interestingly, Nid1-/- but not Nid2-/- mice developed neurological phenotypes such as ataxia and hind limb paralysis, demonstrating a tissue-specific function of nidogen 1 in the PNS. Lamγ1N802S mutant mice mimicked the phenotype of nidogen 1-deficiency. Analyses of sciatic nerves of Nid1-/-, lamγ1N802S mutant, Nid2-/- and wild type mice demonstrate an impaired SC differentiation and defective radial sorting in Nid1-/- and lamγ1N802S mutant mice, which was not observed in Nid2-/- mice. We also show for the first time, that wild type BMs of myelinating and non-myelinating SCs differ in their protein composition. Immunofluorescence analysis demonstrated that nidogen 2 is mainly restricted to the BM of non-myelinating SCs in wild type sciatic nerves. This indicates that nidogen 2 has a more restricted expression pattern in the PNS which might explain the difference of Nid1-/- and Nid2-/-in the observed phenotypes. Moreover, immunoblot analysis revealed that nidogen 2 is re-distributed in the BM of myelinating SCs of Nid1-/- and lamγ1N802S mutant mice. However, this re-distribution of nidogen 2 in the BM of myelinating SCs cannot compensate for the loss of the nidogen 1-laminin γ1 interaction. DISCUSSION AND CONCLUSIONS: Together, these data strongly suggest that SC differentiation is critically dependent on a single protein-protein interaction between the two BM proteins nidogen 1 and γ1 chain containing laminin isoforms.

PA57 Clopidogrel, a selective P2Y12 receptor antagonist and antithrombotic agent, alters adenosine diphosphate (ADP) action on systemic and renal circulation in rats.

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ADP is an endogenous agonist of P^2Y_{12}-R, a member of P^2 purine receptor P^2Y-R subfamily, expressed on extra- and intrarenal vessels and blood platelets. The impact of P^2Y_{12}-R on blood circulation in physiological or pathological states remains unclear; most data based on isolated preparation studies. Earlier research with clopidogrel (Clop) focused on its ability to inhibit platelet's P^2Y_{12}-R. A potential Clop influence on haemodynamic action of ADP was neglected and is the subject of the study.

MATERIALS/METHODS. Our whole-kidney study explored systemic and renal haemodynamics as affected by intravenous ADP in male Sprague-Dawley rats, untreated or Clop treated (20 mg/kgBW/day in drinking water for 2 weeks). Mean arterial pressure (MAP), heart rate (HR) and renal haemodynamics were measured simultaneously under thiopental anaesthesia (100 mg/kg i. P.). Following control (C), ADP in sequential doses of 2-4-8 mg/h/kg was infused, followed by a recovery period. Whole-kidney blood flow (RBF, renal artery ultrasound probe) and intrarenal regional perfusion using laser-Doppler probes placed on the kidney surface (superficial cortex, CBF) or in the outer- (OM-BF) and inner medulla (IM-BF), respectively, were determined. RESULTS. ADP (n=6) induced a dose-dependent decrease of MAP, to 95±4 with the 3rd dose vs 111±2 mmHg in C ($p<0.005$) and a concurrent increase of HR (390±12 vs 340±15 beats/min, $p=0.002$). RBF increased with the lowest dose and remained elevated throughout ADP infusion; it was 9.3±1.1 with 3rd dose vs 8.3±0.9 ml/min/g in C, $p=0.05$). Also CBF increased significantly by 15%. After cessation of drug infusion all the above parameters returned rapidly to baseline. Clop drinking (n=8) attenuated the ADP induced MAP decrease: it remained significant only with the highest ADP dose; the dose-dependent increase of HR was not modified. RBF increase was transient (seen at lower ADP doses only) and CBF did not change. The course of the decrease in calculated renal vascular resistance remained like in untreated rats. In contrast to clear effects of ADP on MAP, HR, RBF or CBF, the medullary perfusion was not affected, similarly under and without Clop treatment. However, after cessation of ADP infusion OM-BF decreased 15±3% in untreated or increased 10±7% in Clop-treated rats; the changes differed significantly from the respective baseline values. Within the IM the post-ADP decreasing tendency (8±3%) seen in untreated rats was reverted to an increasing tendency (7±10%) in Clop pretreated rats. The profiles of OM-BF or IM-BF recovery response for untreated vs Clop pretreated rats differed at $p=0.02$. DISCUSSION/CONCLUSIONS. Evidently, Clop can modulate the extra- and intrarenal vessel tone. We conclude that P2Y12-R contributes to systemic vessel dilatation whereas in the kidney cortical vasodilatation or medullary constriction occur, the latter more distinct in the outer-medullary zone. Partial support: Nat. Sci. Cent., Poland, grant 6442/B/P01/2011/40.

PA58 Refinement of a model of repeated cerebrospinal fluid collection in conscious rats.

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The cannulation of the cisterna magna in the rat is a valuable model for in-vivo sampling of cerebrospinal fluid for studying delivery of new drugs into the central nervous system or disease models. It offers the advantages of repeated sampling without anesthesia-induced bias and using animals as their own controls.
Materials and Methods: An established model was retrospectively reviewed for outcome and it was hypothesized that by refining the method i.e. by 1) implementing pathophysiological based anesthesia & analgesia, 2) using state of the art peri-operative monitoring and supportive care, 3) increasing stability of the cement-cannula assembly, and 4) selecting a more adapted animal strain, the outcome of the model – quantified by peri-operative mortality, survival time and stability of the implant- could be improved and enhance animal welfare. Results: After refinement of the technique, perioperative mortality significantly dropped (7 animals out of 73 compared to 4 out of 322; p=0.001), survival time significantly increased (36±14 compared to 28±18 days; p< 0.001), as well as the stability of the cement-cannula-assembly (47±8 days of adhesion compared to 33±15 days and 34±13 days with two other cement types; p< 0.001). Discussion and Conclusions: Overall, the concept of the 3 Rs by Russel and Burch was successfully addressed and animal welfare improved by: 1) reducing the total number of animals needed thanks to lower mortality or euthanasia due to technical failure and increased usage time frame of the individual rats; 2) improving the scientific quality of the model.

PA59 From animal model to translational strategy: A systematic literature review of animal models for cystic fibrosis.

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Inter-species differences, inadequate research methodology and other factors may contribute to the worryingly low translational value of current animal studies. So-called translational strategies (TS), comprising an integrated approach covering the entire research chain including the patient's perspective, may improve translational success. An important component of TS are systematic reviews (SRs) of animal studies. They help choosing the optimal experimental design for preclinical studies.
Research question We are currently performing such a systematic literature review describing the animal models for cystic fibrosis (CF).For CF, a multitude of animal models is available to the researcher. Preceding narrative reviews have focussed on e.g. genetic mouse models or on specific disease aspects. A complete and structured overview of all available animal models, which can help researchers to choose an appropriate model for their specific research question, is so far lacking. Our SR is meant to answer the question "What are the currently available animal models for CF?" and will also shed light on the sub-questions "What has been measured as a surrogate for CF?" and "Which aspects of the human disease have been modelled?"Methods We developed a search string for Pubmed and Embase based on terms used for cystic fibrosis and standard animal filters. For the purpose of this review, we define "animal model for CF" as animals in which a spontaneous or induced pathological process can be investigated, in which the process, according to the authors, is intended to represent CF in humans in one or more respects. We will exclude studies not addressing CF; studies not in animals (e.g. studies in cells or unicellular organisms and studies describing ex-vivo measurements of tissue dissected from healthy animals), abstracts (without a full description of materials and methods) and reviews not containing new data. Studies in which a pharmacological agent is administered to healthy animals to study ADME or safety will also be excluded. The full protocol for this SR has been posted online (1)Preliminary resultsLiterature searches were performed on28-Dec-2015; from Pubmed 7976 references were retrieved, from Embase 9403. After duplicate removal, 12312 references were imported into EROS for screening of the title and abstract. At the time of submission, 1906 titles and abstracts had been screened by 2 independent reviewers, reaching agreement on inclusion / exclusion for 1885 of them. Title & abstract screening should be finished by 01 April 2016. At the time of the conference, data extraction should be well in progress and the first results will be presented. A descriptive overview of the retrieved models will be given; models will be clustered by induction method, species and strain. This project is funded by the Netherlands Organisation for Scientific Research (NWO_313-99-310)

PA60 A systematic review of the evidence for discomfort due to toe clipping and ear clipping in laboratory rodents.

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Because of their expected impact on animal welfare, toe and ear clipping are generally considered controversial techniques and their performance is restricted or even abolished in many animal laboratories. An abundance of guidelines is available on toe and ear clipping, as well as other methods for individual identification and genotyping. However, these guidelines have, up to now, not been based on a systematic summary of all available evidence.
Here, we provide the first systematic review of the evidence for the effect of toe and ear clipping on rodent welfare. The review methodology was pre-specified in a review protocol, available at www.syrcle.nl. In brief, we performed a comprehensive search in Pubmed, EMBASE and Web of Science, using the search components "toe, tail or ear", "discomfort" and "animal". Data were extracted by one reviewer (MB or FG) and checked by a second reviewer (KW). For studies using a separate control group, two reviewers (KW and MB) independently assessed the risk of bias and study quality using SYRCLE's risk of bias tool. We also assessed reporting of any randomization, reporting of any blinding, and reporting of a sample size calculation as additional study quality indicators. Whenever complete outcome data could be extracted or obtained (i.e. mean, variance and number of animals per group for continuous outcomes, or the number of events and non-events for dichotomous outcomes) we re-analysed the data by calculating the effects size as a standardized mean difference (SMD) or risk ratio (RR), for continuous and dichotomous outcomes, respectively. We will present data on the study quality and risk of bias in this body of evidence, as well as a complete overview of the outcomes assessed and their implication for animal welfare. The results of our review can be used to better inform animal researchers, welfare officers, policy makers and other stakeholders when making decisions on the choice of identification method for rodents.

PA61 Influence of a polyunsaturated fatty acids (PUFA) rich diet and sex on hibernation patterns in the captive Syrian hamster (Mesocricetus Auratus).

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Hibernation is an energy-conserving strategy to escape harsh conditions, such as winter. Hibernating animals suppress their metabolism in torpor, which is alternated with periods of arousal where it normalizes. Previous research has identified that hibernation patterns in marmots [1], either in the wild or captured from the wild, depend on both sex and intake of polyunsaturated fatty acids (PUFAs). Whether these factors also influence hibernation in laboratory bred species is unknown.
Materials and methods Syrian hamsters (Mesocricetus auratus; 30 male, 30 female) were purchased from Harlan Netherlands at 12 weeks of age. Animals were kept for 4 weeks at L:D of 14:10 at 21°C with free access to AM-II control diet and water. Subsequently animals were housed for another 3 weeks with half being fed the AM-II control diet and half AM-II with 3x linoleic acid (LA) with both diet groups containing equal numbers of males and females. Next, animals were housed at L:D of 8:16 for 7 weeks. Thereupon, ambient temperature was lowered to 5 0C and constant darkness was instituted. Passive infrared sensors coupled to a computer system monitored individual movements. Results During 'summer' and 'fall' conditions, body weight of females was consistently and significantly larger than males by on average about 10 g, although the difference reduced in time. Diet did not influence weight in either sex. Upon 'winter' conditions, 46/60 animals entered torpor (76%), of which 36 (78%) survived > 8 torpor bouts. Animals that did not enter torpor were equally distributed amongst sex and diet groups, Animals entered torpor at 11-45 days after switching to winter conditions with the majority of animals entering between days 26-30 (n=9) or 26-40 (n=16). Torpor bout duration increased from 53 to 80 h during 8 bouts, with males showing a larger increase than females. Duration of arousal decreased from 50 to 24 h and shortened more rapidly in males. Sex, diet and body weight did not influence successful entrance into torpor and time till start of torpor. Discussion and conclusions Successful hibernation in the laboratory bred Syrian hamster is considerably lower than reported for animals in the wild or wild-captured animals. Also, quality of hibernation seems decreased, as animals feature both short torpor bouts and extended arousal periods. Possibly, selection against hibernation by commercially breeding facilities induces this trait.In contrast to previous observations in various species, sex and a diet increased in the amount of polyunsaturated fatty acids does not influence torpor bout duration in Syrian hamster. This trait may be related by Syrian hamster representing a facultative, hoarding species, as opposed to e.g. obligatory hibernators that are extensively fat-storing. Nevertheless, sex differences were present, as female Syrian hamsters are heavier throughout any phase of the hibernation protocol than males, regardless of fattening period and diet.

PA62 Alteration of the colitogenic potential in T cells due to Cdcs1.

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The interleukin-10-deficient mouse (Il10-/-) model is suitable to model human inflammatory bowel disease. The colitis severity in this model is influenced by the background strain. A quantitative trait loci (QTL) analysis between C57BL/6J.129P2-IL10 tm1Cgn (B6-Il10-/-) and C3H/HeJBir.129P2-Il10tm1Cgn (C3Bir-Il10-/-) animals was performed and revealed 10 QTL, called Cdcs1 to 10. Cdcs1 on chromosome 3 was identified as the major modifier for colitis susceptibility.
The aim of the study was to investigate the ability of Cdcs1 to modify the colitogenic potential of T cells. Materials and MethodsNaive T cells from B6-Il10-/- and from two subcongenic strains B6. Cg-Il10tm1Cgn MMU3(D3Mit49-D3Mit348)/JZtm (BC-R2) and B6. Cg-Il10tm1Cgn MMU3(D3Mit11-D3Mit348)/JZtm (BC-R3) were isolated and transferred to immune deficient B6.129S7-Rag1tm1Mom (B6-Rag1-/-) mice to cause colitis. Clinical scoring and magnetic resonance imaging (MRI) were performed in vivo and correlated to histological scoring of caecum and colon. Furthermore, a microarray analysis of a naïve CD4+ T cell subset was performed. Differences in gene expression were identified between susceptible and resistant strains and confirmed with qRT-PCR. Results: Depending on the Cdcs1 region, naïve CD4+ T cells are able to induce colitis. The MRI and the histological scoring revealed that Cdcs1 from C3Bir-Il10-/- lead to a more severe phenotype and that the number of transferred cells influences how fast colitis develops. Potential candidate genes for the different colitis susceptibility were identified with a microarray analysis. 47 probes were differentially expressed between the susceptible strains and the resistant strain. Out of these, 10 were located in Cdcs1, which indicates a cis regulation of the genes. Conclusion The experiments showed that it is a promising approach to combine a transfercolitis model and a microarray analysis in order to identify causative genes for colitis susceptibility within Cdcs1.

PA63 The Co-receptor CD14 acts as a protective factor in experimental colitis by improving the intestinal barrier function.

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Introduction: Inflammatory bowel disease (IBD), with the two main forms Crohn's Disease and Ulcerative Colitis in humans, is characterised by relapsing inflammation of the gut. Though its pathogenesis still remains unknown, intestinal barrier disruption likely plays a key role in IBD development. Genetic analyses in the interleukin-10 (Il10) deficient mouse model of IBD revealed Cd14 (Cluster of differentiation 14) as a major candidate gene with likely protective properties.
Materials and Methods: C57BL/6J.129S1-Cd14tm1Smg (B6-Cd14-/-) were investigated in an acute DSS-induced colitis model as well as in a chronic colitis model based on Il10-deficiency analyzing B6.129S1P2-Il10tm1CgnCd14tm1Smg (B6-DKO) mice. A possible protective mechanism of CD14 was explored using transgenic mice (B6.B6D2-Tg(Mt-Cd14)M14S), in which overexpression of CD14 was induced by oral administration of zinc sulfate. The intestinal permeability was analyzed in vivo by measurement of fluorescein-iso-thiocyanate(FITC)-dextran in the urine after oral gavage. Furthermore gene expression of the tight junction (TJ) protein occludin (Ocln) was assesed by quantitative realtime polymerase chain reaction (qRT-PCR). Moreover, inflammatory parameters were characterized by histological examination of the large bowel and gene expression of tumor necrosis factor alpha (Tnfα) and interferon-gamma (Ifnγ). Additionally, immunohistological staining of NFκB-p65 was performed to examine intestinal epithelial NFκB activation. Results: Under steady state conditions B6-Cd14-/- mice showed no differences compared to wildtype controls referring to epithelial and inflammatory functions. However, in the acute and chronic colitis model Cd14-deficient mice showed a significantly

increased intestinal permeability in vivo with significantly decreased Ocln expression levels compared to the control groups. Furthermore, histological examination and proinflammatory cytokine expression revealed a significantly higher intestinal inflammation score in the Cd14-deficient mice compared to the controls in both models of colitis. NFκB-p65 staining indicated a higher NFκB activation in the inflamed intestinal tissue of DSS-treated and Il10-/- mice. However, the intestinal epithelial NFκB-translocation of Cd14-deficient mice did not differ from controls. Finally, overexpression of CD14 was able to prevent the DSS-induced increase of intestinal permeability and lead to significantly increased Ocln expression compared to the controls. Moreover, CD14 displayed a direct protective anti-inflammatory effect through a significantly lower histological score and decreased proinflammatory cytokine expression in the DSS-treated CD14-overexpressing mice. Conclusion: Altogether our results demonstrated that CD14 improves the intestinal barrier function and so plays a protective role in the development of experimental colitis.

PA64 From the operating theatre to the laboratory... And back- the PDTX transplantations in practice.

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Xenografting is a transplantation of cells or tissues from one species to another. To succeed in this procedure, immunodeficient animals are needed. Cell line xenografts are used as a tool for this type of experiments, but they differ significantly from the tumours originally developing in patients. These differences are caused mainly by numerous cell passages, high anaplasia of cells cultured in vitro and lack of connective tissue stroma along with lack of stroma-related interactions.
Materials and methods Since 2014 at Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw using nude mice, we began establishing patient-derived tumour xenograft models focusing primarily on colorectal cancer. Thanks to our collaboration with two clinical departments we obtain a few times a week tissue sections directly from operating theatre. Immediately after taking the sample, tissues are being divided and part of the sample is grafted subcutaneously into mice. The second part of the sample is cryopreserved for further investigation or future transplantations. Results To date we have performed over 50 xenotransplantations of patient-derived tumours of various sites including cancers of gastrointestinal tract, soft tissue sarcomas and melanomas. After exceeding the required size in the first recipients, 10 different colorectal carcinomas are currently being maintained in further passages (1-13). Primary patient-derived tumour tissue and tumour tissues obtained after passages are being molecularly and histopathologically characterized. Discussion Considering the heterogeneous structure of primary tumour tissue in patients, methods that reflect in a better way the original tumour characteristics are needed. Therefore, patient-derived tumour xenografts (PDTXs) play an increasingly important role in preclinical studies of new anticancer therapies. The tumours obtained by that method of engraftment seem to better maintain histological and molecular characteristics and seem to better reflect original environment allowing in some cases preservation of components of human stroma. Additionally PDTX models can be useful in the design of individualized therapies in order to select the agent that shows the highest efficiency in the individual patient.

PA65 Comparison of parenteral buprenorphine and sciatic nerve block for providing postoperative analgesia in a mouse model of femur osteotomy and plate fixation.

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Effective pain management is one of the major challenges in laboratory animals. For provision of analgesia regional anesthesia techniques, the gold standard in human medicine for orthopedic procedures, has not been described in rodent orthopedic models. The aim of the study was to evaluate the efficacy of a sciatic nerve blockade with lidocaine and bupivacaine compared to analgesia with parenteral buprenorphine in mice undergoing experimental femur osteotomy with plate fixation.
Twelve adult C57Bl/6N mice scheduled to undergo orthopedic surgery for an unrelated study were anaesthetised using isoflurane to effect and were randomly allocated to either receive 3 mcg total dose (126 +/- 8 mcg/kg) buprenorphine (group A subcutaneously (SC) or a sciatic nerve block with lidocaine 1% (2 mg/kg) combined with bupivacaine 0.25% (2 mg/kg, (total volume 0.03 mL) (Group B) before beginning of surgery at time 0 h. Postoperatively, both groups received 3 mcg buprenorphine SC at 6 and 12 h and had access to paracetamol 2.1 mg/mL for 3 days in the drinking water. At baseline, 2, 6, 9, 12, 24, 48 and 72 hours after surgery, pain was assessed with a visual analogue scale (VAS) by one observer blinded to treatment identity and gait analysis was performed using a computer-assisted, video-based, unforced walkway system (Catwalk, Noldus AG, Netherlands). Animals bodyweight was recorded every 24 h. We used a repeated measure ANOVA with posthoc Tukey test for data analysis with a significance level of p<0.05.VAS was not different between groups (p=0.4) with changes over time (p<0.0001). Maximal values were 58 out of 100 mm at 6 h postoperatively in group A and B, respectively. Rescue analgesia was not given to any of the mice. Regularity index (%), a measure of interlimb coordination, was neither different between groups (0.35) nor over time (0.41). Duty cycle, expressing stand as percentage of step cycle, was not different between groups (0.09) with significant decreases over time (<0.0001). Comparing left and right hind limb contact intensity per time point in both groups or paw print intensity of the operated leg between groups, there was no difference (p=0.15 for both). Weight loss was not different between groups (p=0.17) with a significant loss in group A at 48 h (p=0.02). In conclusion, this technique of intraoperative perineural analgesia was not inferior to an opioid-based protocol in providing postoperative analgesia in mice for the duration of 3 days.

PA66 Use of animal models for obtaining an idiotypic vaccine for cancer treatment.

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Cancer diseases show a tendency to increase their impact globally; many groups of researchers are working on the collection and development of drugs for treatment; however, extensively drug development depends on animal models, as they are needed to validate therapeutic targets, to anticipate the effects of a drug on a target and to examine potential drug side effects. In this paper we propose aims to show the impact of animal models in the development of a vaccine for the treatment of cancer
Tumor models such as mammary carcinoma F3II, the highly metastatic lung carcinoma 3LL-D122 and MB16 melanoma were standardized and some biomodels as C57BL/6 and BALB/c mice were used in order to evaluate the efficacy and to develop vaccine formulations and immunization schedules of Vaxira, an anti-idiotypic vaccine obtained by the Center of Molecular Immunology. Other models as BALB/c/Xid, BALB/c Nude and Leghorn chickens were used to know vaccine´s mechanisms of action. Furthermore, Sprague Dawley rats and NMRI mice were utilized to evaluate the security of formulation through toxicological studies. All these animal models were of particular use in order to develop and to characterize the Vaxira cancer vaccine, a drug capable of reducing tumor growth and metastasis of non-small cell lung cancer (NSCLC). This vaccine was approved by Cuban regulatory authorities and at this moment it is utilized in Cuban cancer patients. The animal models were essential for obtaining Vaxira vaccine, an original drug for treating human cancer. Biomodels also made possible the development and subsequent use of this vaccine in cancer patients

PA67 Unique Model: Porcine Pulmonary Intravascular Macrophages are prime suspects of triggering CARPA reaction.

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Pigs are recognized as a unique in vivo model based on their sensitivity for Complement (system) activation-related pseudoallergy (CARPA), a life threatening hypersensitivity reaction caused by a wild range of state-of-art nanomedicines. In the process to understand the characteristics of the pigs' unrivalled features for CARPA, our attention turned on pulmonary intravascular macrophages (PIMs), which are present in the lung of species like sheep, rabbit and pig, but are absent from rodents.
Materials and Methods: To clarify the role of PIMs in the process we compared the available literature data with our findings taken from in vivo results, blood tests, histological examinations and a newly developed cell separation technique. Results: PIMs as a lesser-known macrophage subpopulation have unique features that explain of the most severe symptoms – like the dramatic circulatory redistribution - of CARPA in this species. The exceptional features of PIM cells include the followings: 1) strong and constant adherence to the capillary endothel via desmosome-like intercellular adhesion plaques, which secure stable and lasting direct exposition to invaders of the blood stream; 2) their extended, ruffled surfaced glycocalyx membrane engaged in intense phagocytic activity ensures efficient binding and phagocytosis of nanoparticles; 3) PIM cells express various anaphylatoxin receptors, this way Complement activation can trigger these cells; 4) they also express pattern recognition molecules on their surface, whose engagement with certain coated nanoparticles may also activate these cells or act in synergy with anaphylatoxins; 5) their high metabolic activity and capability for immediate secretion of vasoactive mediators upon stimulation explain the rapid - within minutes - elevation of pulmonary arterial pressure, the circulatory disturbance, the respiratory arrest and other robust pathophysiological effects that appears after reactogenic drug exposure. Enzymatic digestion of the adhering structures seems to be an effective method to isolate PIMs and so making them available for further ex vivo examination. Conclusions: These results taken together with the leading symptoms of pseudoallergic reactions, and the possible presence of these cells in human lung suggests that PIM cells may be a potential therapeutic target preventing CARPA.

PA68 Improving transparency on quality and translatability of animal studies using new science driven approach.

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The 3Rs have undoubtedly raised awareness to the way we work with animals in biomedical research. However, implementation of the 3R principles in animal-based research appears to be challenging, and many shortcomings have been identified such as poor experimental design, inadequate reporting and publication bias. To boost improvements in animal-based research, we have chosen a new science driven approach in laboratory animal science, namely the conduct of systematic reviews of animal studies.
Systematic reviews (SRs) are a transparent and thorough method to summarize and synthesize available research evidence. SRs are already standard practice in clinical research and considered the highest level of evidence in the chain of evidence-based research. Even though a substantial number of animal studies are conducted as part of preclinical research, SRs of animal studies are not yet widely conducted. SRs of animal studies have already proven to be of great value, e.g. to provide evidence-base input for the design of new animal studies (including reduction and refinement strategies) and clinical trials. Additionally, SRs provide insights into the translational value of animal research, which should, from both an ethical and scientific point of view, be one of the leading principles in justification of animal use in biomedical research. The methodology used in current reviews of animal studies differs substantially (van Luijk et al. 2014, PlosOne). Only 52.7% of the assessed SRs performed some type of risk of bias analyses. Because only 24.6% of the primary studies applied randomisation en only 14.6% blinding, the risk of bias in the primary studies in these reviews appears to be high. This illustrates the need for better design, reporting and evaluation of animal studies, and additionally also clear guidelines for the conduct of SRs of animal studies, in order to achieve valid scientific conclusions on quality and translation.

PA69 Attitudes and practices of editors of biomedical journals in the reporting and publishing of animal research.

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Scientific journals play an important role in maintaining the quality of the reporting of animal research, a facet of which is spreading knowledge of good practice. Over the last few years, reporting guidelines have been developed and promoted to help editors, reviewers and authors, improve the way animal studies are conducted and reported.
In this study, we aimed to evaluate the quality of reporting of animal studies and assess whether information on good practice in animals use are reported. Two different perspectives were analysed: a) articles reporting experiments that use animals and b) the views and attitudes of editors reviewing animal studies for publication. We ran a systematic review to assess the quality of reporting of articles in a particular area of biomedical research - experiments on murine models of the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS). In this particular area of research, guidelines that aim to standardise the methodology used, mainly for preclinical studies, have been developed1, 2. We reviewed 382 studies published before and after those guidelines were introduced (2007, 2010) to evaluate their impact in practices in animal research of the ALS community. No obvious changes in practice across the years were found, indeed many of the topics described in the guidelines were poorly reported. These results indicate that the ALS guidelines have yet to have a detectable influence on the quality of information reported in animal research, which suggests that other ways to improve this reporting, and hence the associated practices, are necessary. Concerning animal welfare, although reporting of compliance with regulation for animal use and care increased significantly over the years reviewed (2005-2013), in 80% of studies mice had locomotor impairment, but only 10% reported implementation of ameliorative refinement measures. Our results emphasize the importance of, for example, more demanding requirements from the publishers. Editors-in-chief are the principal authority and have autonomy for decisions taken within journals3 . They may also provide guidelines for authors on how to report information on animals used in studies. To understand better the attitudes and practices in editorial decisions over research with animals, we are presently surveying editors-in-chief of various biomedical journals publishing animal research through an internet survey. We intend to address the following questions: a) Do editors have a critical view of how animal studies are designed and reported? b) Is the welfare of animals used in experiments considered in editorial decisions? c) Do editors believe that reporting good practices in animal research is relevant? Preliminary results will be available at the congress.

PA70 From animal model to translational strategy: A systematic review of experimental design in the preclinical and clinical studies of drugs for Rheumatoid Arthritis (RA).

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Inter-species differences, inadequate research methodology, experimental design and other factors may contribute to the low translational value of current animal studies. Besides by the biological inter-species differences, differences in results may also be explained by differences in experimental design between animal and human studies. Systematic review (SRs) of animal studies may shed light on the comparability of experimental design for preclinical and clinical studies.
Scoping We are developing an SR studying experimental designs for efficacy studies in the early- late discovery phase of anti-Rheumatoid Arthritis (RA) drug development. Our scoping searches identified a limited number of RA-drugs tested in humans that lacked clinical efficacy. Result posting for clinical trials has been mandatory (at least in the US) since 2007. As compliance is limited, publication bias could still explain the limited observed attrition of RA-drugs in clinical trials. An alternative explanation is that drugs lacking efficacy for RA drop out from development before the onset of clinical trials. In this context, we want to investigate the quality of protocols used in preclinical classical RA drug trials and compare them with protocols used in clinical trials. Methods A search for all relevant references will be performed in Pubmed and Embase using a search strategy to identify animal and human studies on RA with drugs that are relevant to clinical practice. We created a list of drugs (generic names, brand names and other synonyms) for RA that have been tested in humans by searching medicine compendia and clinical trial registers, and selected the relevant ones based on patient participation. We will extensively compare the design of the animal and human studies and perform assessments of the risk of bias in both. If possible, we will use a meta-analysis to investigate the effects of study design on outcome effect size. Planning Scoping searches have revealed >2500 relevant animal references and >2000 relevant human references. At the time of conference, a preliminary report will be available, and results will be close to publication. These results will be presented at the conference. This project is funded by the Netherlands Organisation for Scientific Research (NWO_313-99-310)

PA71 The value of spontaneous mouse mutations as a source of new animal models.

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Searching for the genotype that underlies a particular mutant phenotype (forward genetics) is a powerful classical approach to discover genes with pleiotropic effects in the mouse. The use of spontaneous mouse mutations in positional cloning projects has been instrumental in the discovery of many genes and gene functions, as well as in the development of new mouse models.
There are several advantages in using spontaneous mutations for this purpose in biomedical research. First, they are produced at virtually no cost and are in general freely available. Second, the researcher is already starting the project with a known phenotype (skin and neurological being the more commonly identified in the animal facilities). Third, spontaneous mutations represent a great variety of molecular events, such as deletions, insertions, and point mutations, generating not only loss-of-function (KO) alleles but also hypomorphic and hypermorphic alleles (with varied effects on protein function). Finally, spontaneous mutations come in multiple backgrounds, including outbred and inbred, which can

potentially uncover new phenotypes due to the effects of modifier genes. In fact, the functions of many genes that were studied using genetically engineered KO mice were significantly underreported, with many phenotypes not detected. In summary, spontaneous mutations help to discover novel genes, genes functions, and novel alleles of known genes (allelic series), and this represent a rich resource for investigating diseases. We will be presenting data on five spontaneous mutations studied in our laboratories through our collaborations between Institut Pasteur and MD Anderson Cancer Center. These examples will clearly show the importance of spontaneous mutations for the generation of new mouse models. These mutations are (i) nackt (Ctslnkt, a mutation on the cathepsin L gene); (ii) barthez and fold (Ass1bar and Ass1fold, mutations on the arginino-succinate synthetase gene); (iii) luca (Zdhhc13luc, a mutation on the zinc finger DHHC domain-containing protein 13 gene-also known as Hip14l); and (iv) ébouriffé (Lrrc8aebo, a mutation on the leucine rich repeat containing 8a gene-also known as Swell1).

PA73 Investigation of Effects of Ghrelin on Skeletal Muscle and Remote Organ Injury Induced by Hindlimb Ischemia-Reperfusion.

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The ischemia-reperfusion (IR) injury on the skeletal muscle is observed with a high mortality and morbidity. Free oxygen radicals and inflammatory responses have crucial roles in IR injury. Ghrelin is a peptide that is synthesized mainly in the stomach and it has anti-inflammatory effects. The aim of this study was to investigate the effects of ghrelin on the skeletal muscle and remote organ injury induced by hindlimb IR.
Materials and Methods: Male adult C57BL/6 mice was used in the study. Hindlimb IR injury was performed by bilateral tourniquet application (2h) upon reperfusion (3h). Three doses of ghrelin were administered (10, 30, 100 µ/kg) to mice intraperitoneally(30min.) before ischemia (G10+IR, G30+IR and G100+IR) and reperfusion onset (IR+G10, IR+G30 and IR+G100). M. gastrocnemius, kidney and lung samples were stained by hematoxylin&eosin (H-E). Histological structures of samples were examined by immunohistochemical methods (IHC, TNF-α). Malondialdehyde (MDA) levels were analyzed by high-performance liquid chromatography (HPLC). Muscle RING-finger protein-1(MuRF-1 is an E3 ubiquitin ligase, which is increased in muscle under atrophy-inducing conditions) levels were analyzed by western blot analysis to examine biochemical changes.Findings: Muscle tissues of IR group had myofibrillar disorganization, degeneration and mononuclear cell infiltration. In Ghrelin group, ghrelin had protective roles in muscle tissues. Peritubular mononuclear cell infiltration was observed in IR group in some locations of kidney and there was a rare tubular atrophy,tubular dilation and vacuolation. A plenty of vasodilation was observed in blood vessels in the cortex and there was an erythrocyte extravasation.Ghrelin had a protective effect on kidney tissues. Regarding lung tissues;IR group showed a mild thickening of the septum and there was a small amount of mononuclear cell infiltration to the parenchyma compared to the sham group.Experimental and sham groups had similar characteristics. TNF-α reactivity was increased in muscle,kidney, and lung of IR group according to sham group. Ghrelin group had low and changing level of reactivity compared to IR group (muscle; $p \leq 0.05$, except IR+G10). When compared to sham group, MDA levels of muscle, kidney, and lung tissues increased in the IR group. Low and changing MDA levels were observed in ghrelin group compared to IR group (kidney; $p \leq 0.05$, except G30+IR, IR+G30, lung; $p \leq 0.05$, except G10+ IR, G30+IR). MuRF-1 levels in muscle tissues of IR group increased compared to sham group. Ghrelin group had low and changing levels of MuRF-1 compared to IR group ($p \leq 0.05$, G10+ IR, G30+ IR). Results: Hindlimb IR caused damage heavily on skeletal muscle, kidney and relatively on lung.Ghrelin exhibited protective effects on these tissues upon IR injury in a dose-dependent manner.
Key words; Ghrelin, Reperfusion injury of the hindlimb, M. gastrocnemius, Kidney, Lung, Mice.

PA74 Does the MHC influence the reproductive outcome in rats?

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A possible influence of the main histocompatibility complex (MHC) on the reproductive outcome in rats was studied. Congenic rat inbred strains are a useful means for this purpose. We compared the reproduction of six congenic LEW inbred strains over one exemplary year. The differences that we found lead to the assumption that the MHC haplotype is important for reproduction.
Studies suggest a possible influence of the main histocompatibility complex (MHC) on the reproductive outcome in mice and rats. Retrospective studies in humans indicate increased spontaneous abortions in relation with autoimmune disorders where the MHC-class II play a role in pathogeneses (1). The comparison of different MHC haplotypes with identical genetic background can give more insight into a possible interrelation of MHC and reproductive outcome. Congenic rat inbred strains are a useful means for this purpose. We compared the reproductive outcome of six congenic LEW inbred strains over one exemplary year. Matherial and methodsRats: Six congenic rat inbred strains (LEW/HanZtm, LEW.1A/HanZtm, LEW.1AR1/HanZtm, LEW.1AR2/HanZtm, LEW.1W/HanZtm, LEW.1WR1/HanZtm) were bred at the Central Animal Facility of the Hannover Medical School under SPF-conditions. Evaluation of the reproductive outcome: The number of live born and weaned pups from every active breeding female was counted weekly. Pre-weaning mortality was determined as the ratio of dead/weaned pups.Statistics: Results were analysed using the GraphPad Prism5 program. We performed one-way ANOVA analysis of variance with post-hoc Tukey´s Multiple Comparison test to determine statistical differences. Differences of the means with p<0.05 were judged as significant. Results and discussion: LEW.1AR1 produced the least number of litter and pups per female. The differences were significant when compared to LEW.1A, LEW.1AR2, and LEW.1W. The number of pups per litter was almost equal for all strains. LEW.1WR1 showed the highest preweaning mortality – LEW the least. The differences that we found lead to the assumption that the MHC haplotype is important for reproduction. Up to 30% of the matings throughout all MHC haplotypes did not produce progeny (2). The reasons are still unknown but are probably based on the genetic background. More studies, with further congenic strain series could help to study the influence of particular chromosomal segments that might be involved in reproduction or other pathophysiological deviants.

PA75 The short lived teleost Nothobranchius furzeri – how a fish can help us to understand vertebrate ageing.

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The demographic change of our society leads to a continuous increase of chronic ageing related diseases. Identification of the underlying biological mechanisms is necessary to allow prevention and treatment of causative physiological changes. The teleost N. furzeri has a very short lifespan of only 3-12 months and develops typical ageing-related lesions and dysfunctions, which makes it an ideal model for intervention studies to modify the onset and development of age-associated pathologies.
In the last decade, we developed the annual African turquoise killifish N. furzeri, into a model organism for ageing studies (reviewed in [1]). The short lifespan is linked to rapid onset of ageing phenotypes at the behavioural, histological and molecular level. The genome is fully sequenced [2] and RNA-seq in the brain demonstrated that the patterns of ageing associated genome-wide transcript regulation are conserved with mammals including humans [3]. Here we show how this fish can be used to address different experimental questions in biomedical research: 1. Germ-line manipulation of gene expression: The availability of the recently published genome of N. furzeri makes it an ideal model for genetic manipulations. The microinjection into the one-cell stage of the eggs, genome editing by CRISPR/Cas9 and Tol2 for transgenesis are well established.2. Somatic manipulations of gene expression: Direct manipulation of gene expression levels in the brain of N. furzeri in vivo can be achieved by intra-cerebroventricular injections. For this, we use microRNA-antagonists or Morpholinos for the knock-down, and expression vectors for over-expression of target genes under study. 3. Longitudinal studies: Because of its short lifespan, N. furzeri allows to perform longitudinal studies in a reasonable time, compared to other vertebrate model organisms, like M. musculus or D. rerio. This is especially of great value for the ageing research, since the integration of transcriptomic and biometric data of a longitudinal cohort allows modelling of complex biological systems. Recently, we were able to identify complex I of the respiratory chain, as being negatively correlated with life-span, based on the results of a longitudinal study of transcriptomic changes at two different time-points in the young adult life of N. furzeri. 4. Pharmacological experiments: To test the role of complex I in regulating longevity, we performed inhibition of Complex I with Rotenone and found an extension of the lifespan and a "rejuvenation" of the transcriptome. Altogether, N. furzeri allows obtaining very detailed information about ageing associated changes on, amongst others, the genomic and transcriptomic level. It can be used for systems biology to understand the complexity of these changes, and is ideal for manipulation of selected genes by in vivo injections of nucleic acids, genome editing, transgenesis, and pharmacological intervention for the identification of new pathways and therapies.

PA76 Spectral analysis of heart rate variability in a rabbit model: the comparison of amiodarone and dronedarone treatments

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Heart rate viability (HRV) is broadly studied to reveal the sympatovagal balance which modulates the cardiac performance. A rabbit model is widely used in researches to represent human heart, although knowledge of rabbit's HRV is still limited. The objectives of the present study were to prove the usefulness of a rabbit model and studied the effects of multichannel blocking agents, amiodarone (AM) and dronedarone (DR) on frequency domain of HRV.
Materials and methods: 16 male NZW rabbits were divided equally into AM and DR groups. The animals were trained to stay in the restrainers 1 hour daily for 7 days before recording the baseline ECG. The rabbits were gavaged with the mixture of AM or DR at dosages of 50 (AM50, DR50) and 100 mg/kg/day (AM100, DR100) with 2.5 mL propylene glycol each for 7 days. ECGs were recorded at the last day of each dosage and the data of 512 NN intervals were computed using the fast-fourier transformation (FFT) algorithm and converted to frequency domain of HRV. The spectral bands, very low (VLF), low (LF) and high (HF) frequency bands were set at 0.0 - 0.04, 0.04 - 0.5 and 0.5 – 2.0 Hz., respectively. All data in the same group were analyzed by using the repeated measures ANOVA and followed by the Student Newman-Keuls Post hoc test. The data between 2 groups at the same dosage were analyzed by using the student t-test. The P value ≤0.05 was considered as a statistically significance. Results: AM100 and DR100 decreased heart rate (HR) significantly compared to the baseline (p =0.041 and p =0.004, respectively). Total power (TP) and LF/HF ratio of AM100 (p =0.014 and p =0.005, respectively) and DR100 (p=0.014 and p =0.001, respectively) were also reduced markedly when compared to the baseline. Additionally, LF of AM50, AM100, DR50, and DR100 were decreased significantly (p =0.035, p =0.0049, p <0.001 and p =0.005, respectively) in a dose-dependent manner, while VLF and HF were not altered by these compounds. Discussion: The rabbit model after receiving AM and DR have shown the negative chronotropic effect. There was the effect of multichannel blocking properties and beta adrenergic blocking activity of these compounds. Decreasing of LF may partially be due to the sympathetic suppression, although some studies suggested that breathing variability might also involve in (1) or mainly dependent on the baroreceptor function (2). However, there is still unclear about the regulation of LF. It could respond to either sympatovagal tone or baroreflex. Additionally, lowering of TP and LF/HF ratio may cause by the decreasing LF component. Conclusions: The effects of multichannel blockers, AM and DR, on HRV in the rabbit model were comparable. These compounds can decrease HR, TP, LF, and LF/HF ratio while VLF and HF revealed unchanging.

PA77 Objective and subjective vision assessment in iodoacetic acid model of swine retinal degeneration.

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The most common inherited disease that leads progressively to loss of night vision up to bilateral blindness is retinitis pigmentosa1 (RP). The iodoacetic acid (IAA) model of swine retinal degeneration2 was created as an alternative model to the costly and time-consuming transgenic approach. The aim of this study was to characterize the IAA model through electrophysiological (electroretinography , Visually Evoked Potential electrophysiology) and behavioral evaluations
Materials & methods Four animals were anesthetized with the following protocol: Tiletamine-Zolazepam 5mg/Kg and sodium thiopental 5 mg/Kg, then were intubated and anesthesia was maintained with isoflurane. Iodoacetic acid (IAA) 12 mg/kg was injected trough the auricular vein. Erg and Vep analysis were performed for both right and left eyes before (baseline) and 4 weeks post IAA injection (post IAA) under general an-

esthesia with the same protocol. Flash and pattern electroretinogram (fERG, pERG) and flash and pattern visual evoked potential (fVEP, pVEP) were recorded to evaluate visual function in terms of rod and cone photoreceptors, ganglion cells and visual cortex activity. Flash stimuli were produced by a stroboscopic lamp in which the light stimulus has a maximum duration of 5 milliseconds and a brightness of 1.5-3 cd s m-2; pattern stimuli consist of a set of horizontal / vertical black and white bars that reverse their position without modification of the global luminance, but with changes due to alternation of the bars with frequency of 2 Hz.For behavior evaluation each animal was tested before (baseline) and 4 weeks after IAA injection (post IAA) in its individual home pen under light or semi-darkness condition. We used a sequence of fear tests 3, the Human Approach (HAT), Novel Object (NOT) and Open Door (ODT) tests specifically adapted to reduce the animals to compensate with hearing and smell. In the HAT, a person walked into the pen and stood still in the middle. In the NOT, a plastic cone was put in the middle of the pen. In the ODT, the door of the pen was opened and it was recorded how fast the pigs left their home penResultsFor both Erg and Vep stimuli, the human ISCEV protocol was successfully adapted for pig. We recorded differences between baseline and post IAA, in particular the cone response (fotopic Erg) was maintained while the rod response (scotopic Erg) was decreased after the treatmentDiscussion & conclusionsThe ISCEV adapted protocol for Erg and Vep stimuli was consistent and gave good quality results concerning the description of the model and future applications (e.g. artificial retina implants). The results are confirming the degeneration of the photoreceptors in particular the cones. Behavioral tests were easily carried out. However, since different responses towards humans were observed in the subjects, we could not set up a standard protocol. We are indeed studying a different behavioral approach to assess vision in pigs

PA78 ICLAS Genetic Monitoring Reference Program.

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The ICLAS Laboratory Animal Quality Network promotes the genetic monitoring of rodents through its Genetic Quality Monitoring Program (GQMP). One of the goals of this program is to provide advice and guidance on genetic quality testing. In order to facilitate this, ICLAS has developed a Genetic Monitoring Reference Program that provides DNA of 12 inbred strains, 4 inbred strains per donor Institution (CIEA, Jackson laboratories, Taconic Biosciences).
Genetic monitoring is critical in order to assure validity and reproducibility of experimental results. One way to assure this is by using genetically defined animals. ICLAS thought it is important to make available DNA standards for use as a cross check reference for laboratories who decide to establish in their animal facilities their own genetic monitoring program. The Genetic Monitoring Reference program has been developed as a concerted effort from three donating Institutions through the ICLAS Quality Network program. This will be a self-sustained program maintained and distributed by ICLAS. A standard purification process has been implemented in all three institutions for DNA isolation. The three institutions interchanged their DNA samples to validate them. We will present the DNA results obtained from validation data from each of the Institutions. We will also present the details on how to request these reference DNA samples.

PA79 A new inflammatory mouse model of infective endocarditis

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Although it is a common and deadly disease, our knowledge on infective endocarditis is lacking. Because of its complexity there are currently no descent animal models. In the standard rodent models of endocarditis, cardiac valve damage is generated by the insertion of a wire through the aortic valve, followed by the injection of bacteria. Nevertheless, 40% of patients with endocarditis have structurally normal heart valves and cardiac valve damage is absent in these patients. Infection in these patients is thought to originate on inflamed rather than damaged heart valves. Material/methods: We developed a new mouse model where endocarditis is triggered by inflammation of the valvular endothelium and not by cardiac valve damage and we tested this model for Staphylococcus aureus and Staphylococcus lugdunensis. In this model we inserted a small catheter through the aortic valve. Through the catheter histamine, a potent endothelial cell activator, was infused during 5 minutes, follow by a 10-minute infusion with S. aureus or S. lugdunensis. Afterwards the catheter was removed. We measured bacterial adhesion to the cardiac valves by using bioluminiscent or fluorescent bacteria or performed gram staining or CFU count of the hearts. The model was compared with the standard model of endocarditis where a small wire was inserted in the carotid artery of a mouse and advanced beyond the aortic valve. The wire was left in place and bacteria were injected 24 hours after surgery. Results: Histology revealed that in the standard endocarditis model infective lesions mainly occur around the inserted wire. This model therefore mimics more a catheter infection, rather than real heart valve infection. However, in our new inflammatory model we were able to induce infective endocarditis in mice, without the presence of any foreign material. By inducing local inflammation of the cardiac valve endothelium we were able to make bacteria adhere to the cardiac valves. The use of fluorescent bacteria allowed us to easily quantify immediate bacterial adhesion on aortic valve sections. This allowed us to identify several key components in the initial adhesion of these bacteria to the cardiac valves. For instance, we identified Von Willebrand factor as a crucial host factor for the development of S. aureus endocarditis. Conclusions: We developed a new model of inflammatory endocarditis for S. aureus and S. lugdunensis. Compared to the standard endocarditis model, this model better represented real cases of endocarditis, because there is no foreign material present at the aortic valve. Moreover, this model gives us a unique glimpse into the early stage of infective endocarditis and can potentially identify the key players in its pathogenesis [3].

PA80 The effect of anti-EGFR therapy in combination with anti-NGcGM3 ganglioside treatment in spontaneous lung metastasis models in C57BL/6 transplanted with 3LL-D122 lung carcinoma.

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The murine models contributed to the understanding of pulmonary metastasis pathogenesis and the development of new therapies. Monotherapies targeting EGFR or the ganglioside NGcGM3 are in relative advanced stages of clinical development at the Center of Molecular Immunology (CIM). Interestingly several reports have suggested a functional relationship between these molecules at the tumor cell membrane, providing a rational for exploring the combinations of immunotherapies targeting them
Introduction: The murine models contributed to the understanding of pulmonary metastasis pathogenesis and the development of new therapies. Monotherapies targeting EGFR or the ganglioside NGcGM3 are in relative advanced stages of clinical development at the Center of Molecular Immunology (CIM). Interestingly several reports have suggested a functional relationship between these molecules at the tumor cell membrane, providing a rational for exploring the combinations of immunotherapies targeting them. Material and methods: We studied the relevance of such combination on spontaneous lung metastases in C57BL/6 transplanted with 3LL-D122-cells (Lewis Lung carcinoma) following the laws of our CIM-IACC (Institutional Committee for the Care and Use of Laboratory Animals). Results: First, anti-metastatic effect of the NGcGM3 vaccine is synergistically increased by the concomitant use of a passive anti-EGFR therapy. Second, our spontaneous lung metastasis model, which resembles the clinical scenery during tumor progression, the combination treatment trigged a strong inhibition of the NGcGM3 ganglioside expression and the interaction between the uroquinase Plasminogen Activator Receptor (uPAR) and integrin a5b1 as signaling pathways relates to escape the immunosurveillance. Discussion and conclusions: This is the first direct evidence of the association between the EGFR and NGcGM3 ganglioside in a spontaneous murine model. Conclusions, this study has strong implication for the development of new combination strategies to enhance the effectiveness of monotherapies.

PA81 The effect of olive extract administration on body and organ weights of adult female ovariectomized rats.

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The aim of this study was to investigate potential changes in the body weights (BW) and organ weights of adult female ovariectomized (OVX) rats under the effect of olive extract administration. Organ and total fat ratios to final body weight (rt%) were calculated and statistically analysed. (All values are presented as mean±SD; only significant p-values are presented.)
Materials and Methods Thirty 16-week-old intact female Wistar rats were recruited for this licensed osteoporosis study. The rats were divided in three Groups: A) Control, B) OVX and C) OVX+Extract (OVX+E). Group C rats were administered the olive extract for 6 months diluted in their drinking bottles after a 2-day rest following OVX and acclimatization to the extract with lower concentration initially. The animals' food intake was restricted according to the Control Group's consumption in order to avoid feeding ad libitum. BWs were weighed every two weeks. After euthanasia, the animals' general condition and their organs were macroscopically evaluated and were weighed immediately after removal. ResultsBaseline BWs were non-significant between groups and at 6 months were Control 338.38±36.32, OVX 383.40±62.21 and OVX+E 400.90±25.6 g (Control vs OVX and Control vs OVX+E both p<0.05). Heart rt% were Control 0.281±0.025, OVX 0.257±0.042 and OVX+E 0.246±0.041%. Kidney rt% were 0.291±0.016, 0.250±0.048 and 0.240±0.028% (Control vs OVX and Control vs OVX+E both p<0.05). Total Fat rt% were 7.737±0.556, 9.038±2.370 and 9.303±0.941% (Control vs OVX p=0.09, Control vs OVX+E p=0.04). Brain rt% were 0.562±0.068, 0.480±0.091 and 0.465±0.068%. Uterus rt% were 0.187±0.034, 0.077±0.047 and 0.072±0.070% (Control vs OVX and Control vs OVX+E both p<0.001). Liver rt% were 2.682±0.244, 2.495±0.374 and 2.476±0.375%. Gastrocnemius rt% 0.521±0.036, 0.437±0.147 and 0.430±0.098%. Conclusions BWs at euthanasia were not significantly different between OVX and OVX+E Groups, indicating that olive extract consumption under restricted food intake according to the Controls' consumption did not cause obesity. Heart, Liver and Gastrocnemius rt% demonstrated non-significant differences between Groups, indicating no effect of ovariectomy or extract administration. Significant differences were detected in Uterus rt% among OVX and OVX+E vs Control, which demonstrate the effect of successful OVX. Total Fat rt% tended to be higher (p=0.09) in OVX and was significantly higher in OVX+E compared to Controls because of the increased abdominal fat in spite of the high BW. Kidney rt% was significantly higher in Controls vs OVX and vs OVX+E due to the Controls' lower BW. Significant differences were not found between OVX and OVX+E Groups, which is a strong indication that olive extract consumption did not have a negative effect on the rats and their organs.

PA82 A Non-invasive DNA sampling method for Genotyping Mice.

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For studies requiring the use of genetically modified animals (i.e. transgenic, knockout), it is necessary to genotype the offspring, in order to determine if they possess or lack the gene(s) of interest. For many years, the standard for obtaining genomic DNA from mice for analysis has been via tissue biopsy, from either tail or ear. Both methods require restraint of the mouse and result in momentary pain and stress experienced by the animals.
Materials and Methods We have successfully implemented the use of fecal pellets from mice to extract, isolate, and amplify DNA for genotyping purposes. Our data suggests that the quality and reproducibility of the genotyping results from fecal pellets and tissue (ear) biopsy are similar. Additionally, the processing time required is comparable between methods. We have used POMC-GFP and GP-Berlin strains for setting up the method. When the animals are ready for genotyping, generally at weaning age, each mouse is placed either in a small clean beaker or in the hands of the caretaker, and allowed to defecate. In most cases, this only takes a few moments. The mouse is then given a temporary ID (tail mark) and returned to its home cage. No restraint or painful procedure is performed on the mouse. The fecal pellet(s) are collected with sterile forceps and placed into sterile micro tubes for processing. The kit used (Bioline ISOLATE Fecal DNA Kit, Cat#BIO-52082) isolates DNA from the pellet(s) in as little as 15 minutes and requires only a vortexer/disruptor and a centrifuge. The DNA is then ready for PCR genotyping. Results Our data suggests that the quality and reproducibility of the genotyping results from fecal pellets and tissue (ear) biopsy are similar. Additionally, the processing time required is comparable between methods. DiscussionAnalysis of fecal samples has several clear advantages including its non-invasive nature and

the possibility for repeated samplings if needed. It is a simple and fast method and although the processing cost per fecal sample is more than cost when using the tissue biopsy, European regulations states that wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure. Furthermore, any reduction in stress to experimental animals should have positive scientific implications. An estimated 1000 mice require genotyping annually in our facility, based on historical breeder programs and litter sizes. Conclusions It is possible to use feces as high-quality genomic DNA material for routine genotyping of genetically modified mice.

PA83 Lurking in the shadows: how animal facilities affect your research.

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There is a substantial use of wild type and, moreso, genetically engineered mice in biomedical research. Ideally, the experiments should conform to the 3Rs (Russell and Burch, 1959) and the results should be able to be translated to the human field.
It is well-established that standardization of experimental conditions is paramount for reproducibility of results. However, many factors in the animal facility can influence the experimental results including the microbiota and infectious agents, housing systems, type of feed, treatment of the water, and handling practices. The aim of this talk is to give an overview of these confounding parameters with some examples, thereby aiding in a more efficient planning and execution of experiments.

PA84 Systematic reviews of animal studies; missing link in translational research?

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Standardization is assumed to guarantee precision and reproducibility of animal experiments. However, evidence indicates that standardization compromises the external validity and reproducibility of experimental results, whereas systematic variation of experimental conditions may improve external validity and reproducibility1, 2. Here we used published data from single-lab studies to examine whether multi-lab studies may improve the external validity and reproducibility of results.
Data were drawn from the CAMARADES database on the effects of hypothermia on stroke3. The database contained 50 independent comparisons of infarct volume between treatment and control groups that met our inclusion criteria. These 50 comparisons were sampled to create a matrix containing 12 replicate studies each of single (1 lab), duplet (2 labs), triplet (3 labs) and quadruplet (4 labs) studies, respectively, and we used the parameter estimates (μ, s) of control and treatment groups from the original comparisons to generate sample values (N=12). This procedure was repeated 10'000 times for simulation. For each single or multi-lab study, effect sizes and their 95% CIs were calculated using a random effects model. Results: Variation of effect size (CI) across replicate studies was largest in single-lab studies and decreased with the number of labs per study. Although, single-lab studies were more precise (smaller within-study variability), they were less reproducible (larger between-study variability) compared to multi-lab studies. Thus, while only a few single-lab studies (15.7%) included the overall effect size (reduction of lesion volume by 49.5%) in their CI, this proportion increased to 25.7% in duplet studies, 31.1% in triplet studies, and 34.2% in quadruplet studies, respectively. Conclusion: These results confirm earlier findings1, 2 indicating that standardization of study conditions reduces external validity and impairs reproducibility of experimental results. Thus, systematic variation of study conditions (heterogenization), as in multi-lab studies, is needed to generate effect size estimates that are externally valid and reproducible across independent replicate studies.

PA85 Pharmacokinetics of iohexol in urine of mice following a single oral administration: identification of the optimal time-points for urine collection during the development of an intestinal permeability test in mice.

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Iohexol is a radiographic contrast medium that has also been successfully used as an in vivo intestinal permeability marker in humans, dogs, horses and rats. In vivo evaluation of intestinal damage in mice is rare, but development of a minimally-invasive test for evaluation of intestinal permeability in mice would refine and reduce this mouse model.
Objective: The aim of our project is to identify a suitable marker for the development of an in vivo intestinal permeability test in mice, and the aim of this specific study was to determine the time-course of iohexol in urine of mice after a single oral administration of the molecule. Materials and methods: Female Hsd:AthymicNude.Foxn1nu mice (n=15) were administered a single oral dose of 10 ml iohexol/kg before the animals were randomly placed in individual metabolic cages for urine collection during a seven-time point periods (2, 4, 6, 12, 15, 18 and 24 hours), starting from 7pm to 7am. The amount of urine collected in Eppendorf tubes was gradually measured and frozen in -20Cº before analysis using Enzyme-Linked Immunosorbent Assay (ELISA), specifically the FIT-GFR Iohexol Kit. Results and Discussion: The results of the study showed that urinary excretion of iohexol from a healthy group of mice progressively increased to up reaching the first peak (4 hours), thereafter decreased until the second peak (12 hours), and finally declined gradually up to 24 hours. These two highest peaks of iohexol at 4 and 12 hours may indicate the peak iohexol concentration in small and large intestine, respectively. Interestingly, after 12 hours of confinement all mice (100%) delivered urine into the metabolic cage. Conclusions: We conclude that iohexol as an oral biomarker for use in in vivo intestinal permeability tests in mice shows potential value for evaluation of intestinal damage in several mouse disease models and can help reduction and refinement in a considerable number of mice used in research.

PA86 Cryopreservation of mutant mouse lines: Improvement of embryo production and influences on the progeny after recovery.

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Genetically modified mice are unique mutants with an enormous scientific potential and rapidly increasing numbers. Small populations, continued danger of loss, often a small breeding success, the need to keep them in stock even if they are out of experimental use and frequent interchanges between different facilities are issues when dealing with these mutants. Cryopreservation of pre-implantation embryos is a valuable alternative to breeding. Hence, strict assessments are mandatory.
Aim of the cryopreservation is the secure recovery of the line also after years of storage. Following sufficient cryopreservation the breeding of a line can be discontinued. Cryopreserved samples can be stored at -196°C unlimited. Prerequisites for an economic cryopreservation are high embryo-yields prepared from donors and a high recovery rate following revitalisation. In addition to standardized housing conditions and superovulation-protocols, the optimum age of the embryo-donors of an individual strain must be specifically determined. Housing at 24°C increases the embryo-yields. Diets for laboratory animals are often produced from phytoestrogen containing soy, our data show that feeding the donor animals with phytoestrogen poor diet is much more efficient compared to phytoestrogen containing, soy based diet. The production of two-cell embryos is more economic than that of eight-cell embryos. However, the capacity to develop to the next embryonic stage was dramatically reduced when frozen/thawed two-cell embryos were compared to eight-cell embryos. Following embryo transfer the sex ratio became uneven and more males were delivered. This effect might be due to the procedures animals and embryos were subjected to. The revitalization rate of cryopreserved embryos stored in-house or imported was compared. The storage period did not affect the revitalization rate, whereas the recovery-rate of imported and shipped embryos was significantly reduced. This might be due to handling and shipment of the cryopreserved samples. The genotypes of genetically modified pups received following revitalization were slightly smaller than expected according to the Mendelian laws. Revitalized embryos do not undergo increased apoptosis-rates if compared to non-cryopreserved embryos. Mouse pathogens were never detected in cryopreserved specimens; however, there might be limits in the assessment assays. Since environmental organisms were found in the permanent freezers and for safety reasons, foster mothers and revitalized pups should be housed in intermediate facilities. Their health must be assessed before importing into the target facility. These data show that many parameters can influence the production of animals when using (frozen/thawed) embryos. These parameters need continuous surveillance.

PA87 Extracorporeal circulations maintained by a heart lung machine in a large animal model in pigs, a model for research and training.

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Experimental surgery in pigs is an important field of research to investigate new surgical techniques and to improve skills of surgeons. Pigs (60-80 kg) are comparable to human in their size and organs (heart and lung) and thus are a perfect training object. Although surgeons can improve their skills in dummies, it will be never as good as under realistic conditions with live tissue.
But there are some surgeries like heart and lung manipulations, where bloodstream has to be maintained by extracorporeal circulation. Therefore a heart lung machine is connected to the big vessels to supply oxygen and preserve blood pressure. The arterial line is inserted in the aortic root and the venous line through the right auricula in the right heart. The machine is started and the aorta is crossclamped caudal of the inserted catheter. Heart arrest is induced by hyperkalemic solutions infused by a coronary sinus catheter. Thereby optimal conditions for complex cardiac surgeries are preserved. Blood temperature and flow can be regulated, whereas it is often necessary to increase blood pressure by vasoconstrictive medication. In case of non-acute experiments it is possible to wean pigs of the heart lung machine and wake them from anaesthesia. After this procedure they need a period of post-operative intensive care including support in circulation regulation, sufficient analgesia and antibiotic therapy. The cardiopulmonary bypass model in pigs is a recommendable model for difficult questions in cardiac and thoracic experimental surgery where an extracorporeal circulation is necessary. Furthermore it is a chance for surgeons to improve their skills and to establish new surgical technics without threatening human life. This poster presents the practical use of the cardiopulmonary bypass in pigs, gives an overview about expectable problems and their approach.

PA88 Micro Sampling and the 3R's: The use of Capillary Micro Sampling (CMS) and EZ-Spot® dry whole blood spot (DBS) technologies for routine serosurveillance and biomarker analysis.

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Micro sampling techniques including CMS and EZ-Spot® DBS are important tools for routine serosurveillance of rodent colonies as well as analysis of biomarkers in discovery studies. Both uses a drop of whole blood or less and are alternative to submitting rodent serum for routine serology screening and/or biomarker profiling in discovery studies. Data shows that in accordance with the 3R's principle the overall number of animals used in the studies can be reduced.
Material and methods: EZ-Spot® cards were used to collect a drop of whole blood sample. Cards were punched and shaken with buffer to elute antibodies. LOD comparison of eluted antibodies vs. serum was done by doing endpoint MFIA titrations (up to 3,125-fold dilution) on serial 5-fold dilutions of mouse and rat monospecific antisera. Two analysts each performed triplicate MFIA runs of 16 sera (rats and mice each) and matching DBS to determine sensitivity, specificity and reproducibility. Microsampling efficacy of hematology and chemistry parameters for various species was tested on whole blood and sera, respectively. These were collected in normal tubes and/or CMS, and by testing their dilutional linearity by serially diluting them in 10% increments. In a mouse study serum corticosterone levels at several time points (1h-24h) were compared to those collected using CMS and DBS techniques. Results: None of the 2,496 MFIA performed in six runs of 16 SPF rodent sera gave positive reaction with EZ-Spot® eluates and matching antisera thus showing high specificity of the assays. Of the 720 immune sample-assay combinations expected to yield positive results, 714 (99.2%) of the sera and 702 (97.5%) of the DBS eluates gave positive net scores. Five-fold dilution linearity of WBC, RBC, HGB, HCY, PLT hematology parameters was achieved in multiple species including rats and mice. Similar five-fold dilution linearity of most of the clinical chemistry markers was achieved including CHOL, TRIG, HDL, LDL and FFA in hamster serum. ELISA serum corticosterone levels

in the mouse study were comparable to CMS and DBS levels with +/- 20% recovery. Discussion and conclusions: Sample data on EZ-Spot® vs. serum have comparable analytical and diagnostic sensitivity and specificity, reproducibility and ruggedness. Immune biomarkers are important indicators and regulate many processes in progression or state of diseases. Their profiles can provide necessary drug efficacy and/or toxicology information in multiple stages of drug development. Micro sampling techniques can be useful tool for studies especially in small animals where amount of blood and/or serum is limited and extra safety parameters can be added for testing. Hematology and clinical chemistry data confirms that smaller volumes (fivefold less) can be used in studies without comprising the results. Data also confirms that in accordance with the 3R's principle we can also reduce overall numbers of animals in the studies.

PA89 Refinement and Basic research – A combination study in a mouse-osteotomy model to reduce lab animal usage.

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Fracture healing disorders occur in approximately 10% of human patients and cause severe pain and reduced quality of life. To develop and test new therapeutic strategies, the mouse is a frequently used laboratory animal in bone healing research. However, the identification and assessment of pain, stress and strain in the mouse is still challenging. In addition, the selection of analgesics in bone healing models is restricted due to potential interfering properties of anti-inflammatory drugs.
Here, we evaluated two commonly used pain management protocols, Tramal (0,1 mg/ml and 1 mg/ml) and Buprenorphine (1 mg/kg) administered with the drinking water, for their efficiency and side effects on experimental readout in a mouse osteotomy model. Weight, water and food uptake, nest building activity and other behavioural parameters of wellbeing as well as pain signs (Mouse Grimace-Scale, clinical scoring) and hypersensitivity (Hotplate) were assessed. In order to evaluate the influence of pain management on bone healing and experimental readout, we performed µCT analysis and histological staining of the osteotomized bone to gain highly important data for experimenters working in the bone research field. Refinement studies are often performed by lab animal scientists and separated from basic research approaches. Therefore, the translation of these results back to the basic research is challenging due to the unawareness on refinement approaches of the basic research-oriented experimenter. In order to enhance the knowledge on refinement options in basic research studies and to effectively reduce lab animal usage, we conceived a refinement study embedded in a basic research study in the mouse osteotomy model to show the possibility to combine both studies. Therefore, the control animals (without osteotomy) were reused in the basic study as well as the results of the operated animals. At the end of the study, we aim at preparing standardized score sheets and recommendations for an evidence-based pain assessment procedure in bone-linked mice models. Here, we will present preliminary results of our refinement study and present our combining concept to provide a possibility to reach a wider community with results in the field of refinement studies.

PA90 The effect of cage height on bone density of growing Wistar rats

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Several welfare guidelines and legislative requirements regulate cage dimensions used for the housing of laboratory rats. Research has shown that increased dimensions and complexity have varying effects on their overall health (1, 2). The aim of the present study was to investigate the potential beneficial effect of daily low impact exercise with the use of two-story cages on bone mineral density (BMD) of growing Wistar rats.
Materials and methods Forty 30-days-old Wistar rats (20 males, 20 females) were randomly housed in either common cages (CC, 18 cm height) or two-story cages (Double Decker/DD, 40.4 cm height, Tecniplast) per sex in 4 Groups: Group CCM (CC males, n=10), Group CCF (CC females, n=10), Group DDM (DD males, n=10) and Group DDF (DD females, n=10). Dual X-ray absorptiometry for measurement of their proximal tibial bone density was conducted at 2, 4 and 8 months of age under brief anesthesia. As this study did not include invasive procedures, the animals were used in other protocols after its end. Results BMD measurements of the proximal tibia at 2 months of age were similar in all 4 Groups. At 4 months of age, BMD of CCM and DDM rats were statistically significantly different with values of 0.352±0.04 and 0.394±0.03 mg/cm2 respectively (p=0.04), however at 8 months their values were 0.428±0.03 and 0.464±0.06 mg/cm2 respectively (p=0.10, non-significant/NS). At 4 months of age, BMD values of CCF and DDF rats were 0.381±0.05 and 0.420±0.03 mg/cm2 respectively (p=0.08, NS), and at 8 months were 0.439±0.04 and 0.453±0.03 mg/cm2 respectively (p=0.47, NS). By analyzing together CCM+CCF versus DDM+DDF, differences between caging systems were more prominent at 4 months with p=0.008 and at 8 months p=0.075. All results are mean values ± standard deviation. Discussion Our results indicate that for male rats, housing in two-story cages produced an increase in their bone density throughout the growth period observed, which was statistically significant at 4 months of age. For female rats, housing in two-story cages produced a mild increase in their bone density particularly early in time which indicated a tendency towards statistical significance. The difference in caging systems was statistically more pronounced when analysis was conducted with both sexes due to the increase of subject numbers. Conclusion Daily mild exercise of growing rats by using a two-story cage, in addition to providing an increased caging system complexity, with more stimuli and a wider selection of space for activities, had a beneficial effect on their bone density.

PA91 Direct PCR on hair follicles: a rapid, inexpensive and non-invasive genotyping technique for large mouse colonies.

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Genotyping is a routine technique in genetically modified mice. FELASA guidelines recommend reducing discomfort to animals when obtaining DNA samples for genotyping. However, in practice, scientists still use traditional invasive methods such as tail biopsy, ear punching or blood samples, and DNA purification/amplification can be long and expensive. We describe a simple non-invasive technique to genotype large mouse colonies by direct Polymerase Chain Reaction (PCR) on hair follicles.
We chose to use hair follicle samples to genotype the 15 mouse strains in our laboratory because of the special practicability of this technique and its respect of the 3Rs (Replacement, Reduction, Refinement). In this technique, we directly placed hair follicles in a simple alkaline digestion buffer. PCR was performed directly on 1 µl of this sample preparation. This step avoids DNA purification and represents a real gain of time as it

takes as little as 2 hours from the sampling to the PCR results. Members of all scientific teams in our laboratory (12 persons) have been trained in its use. In order to avoid all cross-contamination, a procedure was developed for collection of hair samples, guided by a risk assessment protocol. The accuracy of genotyping and absence of cross-contamination was checked by using randomly selected tail samples as controls for each mouse strain. No cross-contamination has ever been observed. This technique has been in use in our laboratory for over 8 months now, and has been tested in 1583 animals from 15 mouse strains and 19 research projects. All scientists have accepted the new technique for genotyping without reserve, as it represents less discomfort for the animals, and is quicker, less expensive and as accurate as traditional methods.

PA92 STR (Short Tandem Repeats) marker analysis based on tetranucleotide repeating units allow for genetic characterization of mice to a new level of differentiation and for genetic monitoring and control of strains, substrains and even individuals.

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Unclear or misclassified genetic background of laboratory rodents or a lack of strain awareness causes a number of difficulties in performing or reproducing scientific experiments. Until now, fine definition and genetic differentiation between strains and substrains of inbred mice has been a challenge. STR markers comprising of tetranucleotide repeating units allow for identification and characterization of mouse strains and substrains to an unprecedented degree of differentiation.
METHODS: A set of highly informative STR (Short Tandem Repeats, microsatellites) markers covering the 19 autosomes as well as X and Y chromosomes have been identified. Several strains and substrains of C57BL/6 have been analyzed by employing these markers using multiplex PCR and capillary electrophoresis. RESULTS: The results indicate that strains and substrains can be differentiated with a large number of informative markers: Up to 50% out of more than 250 markers differ between C57BL/6 J and N substrains. Individuals of the same subgroup J or N, provided from different commercial breeders exhibit considerable differences in their STR profiles. The level of genetic difference between particular substrains perfectly matches the genetic tree of C57BL/6. This means that STRs can be utilized to describe and to monitor genetic drift.Y-chromosome specific STR-markers allow for the generation of haplotypes that can be used to derive an unequivocal signature for each of the substrains of C57BL/6 or other inbred mice strains.Our data demonstrate that even siblings within inbred strains can be differentiated and allocated to parent couples using STRs. No individual has proven to be genetically identical with another. This finding is in stark contrast to SNP data, indicating that individuals of inbred strains are genetically identical. The large number of informative markers provides the opportunity for genetic background determination of strains or substrains and individuals of unknown pedigree as well as speed congenics projects for back-crossing from mixed genetic background to well defined genetic background, monitoring of genetic drift. Application of the STR-marker set is not restricted to C57BL/6, it is extremely flexible with regard to combinations of strains or substrains of any inbred mouse strain. OUTLOOK: Which level of genetic variability is acceptable and required to guarantee validity and reproducibility of experiments, in order to control cost and effort, within the breeding process, still needs to be defined.

PA94 A novel approach allows repeated rapid arterial blood sampling in rodents and results in a reduction of animal use in quantitative PET imaging.

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Small animal Positron Emission Tomography (PET) is an established method to visualize specific molecular targets using radiolabeled compounds. Various forms of data analysis are used to quantify binding of a radioactive probe to its target, Logan graphical analysis is a commonly applied tool which requires rapid arterial blood sampling during the scan. We have developed a method which allows re-use of rats, repeated PET scanning and blood sampling (which were not possible until now).
Materials and methods: Male Wistar rats were catheterized via a superficial branch of a femoral artery. To evaluate the reproducibility and variability of repeated PET scans involving this novel surgical procedure, a 60 min dynamic PET scan of the brain was acquired using the adenosine A1 receptor ligand 11C-MPDX (39±18 MBq). Arterial blood samples (0.1-0.15 ml) were collected at 15 different time points during the scan. Twenty-five µl of whole blood was collected from these samples, and plasma was collected from the remaining blood by centrifugation to acquire a plasma time-activity curve (TAC). After this initial scan, the artery was closed and the wound sutured. Subdermal Marcaine 0.5% (max. 2.5 mg/kg) was applied as analgesia. Rats were checked daily for weight loss and signs of discomfort. Exactly 1 week after the first scan, the surgical procedure was repeated and a second PET scan with 11C-MPDX (26±13 MBq) was made with rapid arterial blood sampling. Plasma time-activity curves (TAC), whole blood radioactivity and metabolite data were used as input functions for Logan graphical analysis of 11C-MPDX binding, to calculate tracer distribution volume (VT) in the brain. Results: Surgery was successful in 90% of the cases. Wounds closed and body weight was recovered within 2 days with no signs of discomfort. PET images showed strong uptake of 11C-MPDX in hippocampus, striatum and cerebellum. The relative difference and test-retest variability between scan 1 (test) and scan 2 (retest) were in all brain regions < 5%, except for olfactory cortex, hypothalamus and bulbus (5-10%). The reliability of the measurements between and within subjects, expressed as Interclass Correlation Coefficient (ICC), indicated a high agreement between test and retest, with values between 0.69 and 0.98 for all regions. Reduced values for ICC were observed in regions with low receptor densities or very small volumes (< 60 µl). Discussion: Although the new technique allows repeated use of animals it has some limitations. The technique can only be applied in rodents with a similar (or larger) size than rats. Mice are too small for the drawing of many blood samples. Large rodents can be used only 2 or 3 times since the catheterized artery suffers some damage and can therefore not be re-used. Conclusion: This new surgical procedure allows re-use of animals (2-3 times) in quantitative PET imaging including rapid arterial blood sampling, with a high test-retest reproducibility.

PA95 Animal use replacement by human atherosclerotic carotid plaques in Positron Emission Tomography (PET) imaging of vulnerable plaques.

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Rupture of vulnerable carotid atherosclerotic plaques is the primary cause of acutevascular disease. Detection of vulnerable plaques before rupture can be life-saving. Positron Emission Tomography (PET) uses radioactive probes to identify vulnerable plaques. ApoE -/- mice, prone to develop atherosclerosis, are commonly used to develop such probes. We now show that ex vivo imaging of human atherosclerotic plaques can be used as a replacement for rodent models in radiopharmaceutical development.
Materials and methods: To determine if human atherosclerotic carotid plaques can be used as replacement for rodent models in the development and evaluation of radiopharmaceuticals, carotid plaques were scanned with fluorodeoxyglucose (FDG), a metabolic marker for macrophages. The presence of macrophages in plaques is known to cause vulnerable plaques. Patients with significant carotid atherosclerotic stenosis underwent carotid endarterectomy (CEA) at the Department of Surgery. Carotid plaques were removed and transported in phosphate-buffered saline (PBS) to the imaging facilities. The plaques were then incubated in approximately 50-100 MBq of FDG for 60 minutes by 37 0C. After incubation the resected plaques were flushed with PBS and were subsequently stored in a humid environment during imaging procedure to prevent dehydration. Plaques were scanned in a small animal PET scanner for 60 minutes followed by computed tomography (CT) for anatomical reference. After the scans the plaques were frozen or embedded in paraffin for immunohistochemistry. CD 68 immunostaining for macrophages was used and correlated with the PET scan findings. The PET and CT images were fused and analyzed by drawing volumes of interest around the targeted areas to determine the uptake and distribution of FDG. Results: The PET images showed heterogeneous distribution of FDG in the plaques. High uptake of FDG indicated intense macrophage infiltration, which was confirmed with a positive correlation between the distribution of macrophages and the FDG uptake (r = 0.68, P < 0.01). Low uptake of FDG was associated with stable plaque calcification. The calcification level was inversely correlated with FDG (r = -0.84, P < 0.001). Discussion: FDG incubated plaques may be an alternative for rodent models in the development and evaluation of radiopharmaceuticals. This experiment led us to further ex vivo tissue use in the evaluation of radiopharmaceuticals such as 89Zr-Bevacizumab (marker for the release of vascular endothelial growth factor), 18F-RGD (marker for $\alpha v\beta 3$ integrin) and 18F-NaF (marker for microcalcification) and probes for optical imaging such as MMP-9 (marker for matrix metalloproteinases). Conclusion: Ex vivo imaging of human atherosclerotic carotid plaques is an alternative for atherosclerotic animal models in the development and evaluation process of new radiolabeled compounds for the identification of vulnerable plaques.

PB1 Administration of an Experimental Chronic Wasting Disease Vaccine to Thirty Wapiti Heifers Followed by Challenge and Evaluation over a Twelve Month Period in BSL3 Containment.

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Research at VIDO-InterVac focuses on vaccine development to mitigate the impact of infectious diseases on human and animal health globally. Researchers here have developed a novel vaccine targeting the prion believed to be the causative agent of Chronic Wasting Disease in cervids (deer and wapiti). To evaluate this vaccine in the target species, thirty vaccinated female yearling Wapiti were maintained in the InterVac Level 3 facility where they were challenged and monitored for twelve months.
The emergence of Chronic Wasting Disease (CWD) in Canada has had a devastating effect on the farmed elk/deer industry in western Canada. The spread of this disease through the wild populations of cervids (deer, wapiti, moose and caribou) not only threatens a valuable natural resource but also impacts a major traditional food source of first nation populations. In the absence of any treatments, there exists a pressing need for an effective vaccine to prevent or minimize spread. Vaccines are the most effective tool for the control of infectious disease in both animals and humans. Vaccine formulation is particularly challenging for prion diseases like CWD because the causative agent results from mis-folding of a normal self-protein (PrPC) into the pathological and infectious conformation (PrPSc). Research at VIDO-InterVac is focused on the development of a novel vaccine targeting this anomalous prion. To this end thirty recently weaned Wapiti heifers (hinds) were vaccinated twice at a 45 day interval and subsequently housed for one year in the Level 3 Containment facility at InterVac for challenge and subsequent monitoring.VIDO-InterVac's BSL3-Ag containment facility has 18 animal rooms each having over 50 square meters of floor space. They can be configured so that multiple rooms can be utilized as a single space. In this trial three connecting rooms held ten animals each and a fourth room contained a single purpose-built handling system composed of six 2.3 square meter cubicles leading into a manually operated padded squeeze. The challenges of handling non-domesticated animals of 100 to 200 kg body weight were considerable. They ranged from self-injury due to fear-crowding and stepping on one another's lower limbs to curiosity-associated mischief such as discovering how to open gate latches. We will show how the design of the facility allowed these animals the opportunity to express many of their normal behaviors. This poster will depict some of the difficulties encountered in this trial and their solutions. Understanding Wapiti behavior by the animal care staff was critical to the success of this experiment.

PB2 Young to middle-aged dogs with high Abeta levels in CSF are impaired on learning in standard cognition tests.

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Understanding the relevance of changes in Alzheimer's disease biomarkers that occur before the pathology becomes evident, can contribute to the development of a treatment for Alzheimer's disease. A longitudinal follow-up of an animal species with a similar amyloid pathology in the brains as in humans may contribute to this research.
Amyloid plaque formation is one of the two main neuropathological hallmarks of Alzheimer's disease in humans. Dogs are similar to man with respect to amyloid precursor protein (APP)-processing and age-related amyloid plaque deposition. Dogs also are used as a natural model of age-dependent cognitive dysfunction. In our colony of beagle dogs Aβ-concentrations in cerebrospinal fluid (CSF), sampled in awake animals from the lateral ventricle, were regularly measured over a period of years. We identified dogs showing low or high Aβ42 levels and formed two groups of ten animals each. The age of the animals, which ranged from 2-8 years, was comparable between both groups. Since dogs normally start to develop amyloid plaques from an age of 9-10 years onwards, these dogs are assumed to have no or minimal amyloid plaque formation. The cognitive performance of these dogs was evaluated in standard cognition tests such as object discrimination learning, reversal learning and delayed non-match to position (DNMP). A difference in learning performance was observed between dogs with low and high CSF Aβ concentrations. Our data suggest that high levels of Aβ in young to middle-aged dogs might contribute to learning impairment prior to amyloid deposition. Further experiments are needed to investigate whether there is a causal link between high levels of CSF Aβ and cognitive performance in young to middle-aged dogs as well as the longitudinal sequalae of these differences with respect to disease progression.

PB3 Bedding for primates in a BSL 3 facility

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In nature, macaques spend almost half of their awake time foraging and this behaviour is difficult to satisfy in any research facility, but especially in experiments requiring higher biosafety levels. The aim of this study was to investigate how to maintain a high safety level for staff working in a BSL 3 facility, while still optimizing the opportunity for the primates to express their natural behaviours.
Material and methods The behavior of 6 female Cynomolgus macaques was observed in five minutes' intervals for a period of …. The time the monkeys was inactive, foraging or grooming was registered, first in a cage with no floor bedding, then with bedding and then again without bedding. In all three cases the animals were given enrichment items, such as tennis balls or Kongs. The cage was also enriched with a foraging mat, puzzle feeder, challenge ball and an enrichment cup filled with wood shavings and treats. Results Our study showed that the time spent foraging was approximately doubled in the cage with floor bedding. This means that the monkeys displayed a much higher degree of natural behaviors in the cage with bedding. From the care-taker's perspective, the addition of bedding did not change the overall time spent cleaning and taking care of the animals. Although changing bedding is time consuming, this was done only once a week. The other days of the week, the daily cleaning was much faster. Working with extra thick protective gloves for prevention from injuries was not considered a problem by animal technicians. Discussion and conclusion The results clearly show that with very little efforts and no extra time consumption you can considerably increase the wellbeing of the animals. Expressions of natural behaviors has been shown to decrease stress and stereotypical behaviors, factors that may cause unwanted variances in a number of biological variables. In conclusion, making the small effort of adding bedding to the monkeys will in turn result in much more reliable research results.

PB4 Assessing the 3Rs Value of Automated Positive Reinforcement Training for Laboratory Non-Human Primates

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Behavioural training through positive reinforcement techniques is well recognised as a refinement to laboratory animal welfare. However, exclusive use of positive reinfocement training (PRT) techniques - without the use of negative reinforcement, positive punishment or negative punishment - can require expertise and time that animal care staff or research personnel do not have the confidence or ability to provide. Automated PRT systems may be a solution to this problem.
Behavioural neuroscience research requires subjects to be trained to perform repetitions of specific behaviours for food/fluid reward. Some animals fail to perform at a sufficient level, limiting the amount of data that can be collected from each animal and increasing the number of animals required for each study. We have implemented automated positive reinforcement training systems (comprising a button press task with variable levels of difficulty using LED cues and a fluid reward) at the breeding facility Centre for Macaques (CFM) and Newcastle University Comparative Biology Centre (CBC) to pre-screen animals for selection and refine training protocols. We found that animals learned 1- and 4-choice button tasks within weeks of home enclosure training, with some inter-individual differences. High performance levels (~200-300 trials per 60 min session at ~80% correct) were obtained without food or fluid restriction. Moreover, training quickly transferred to a lab-based version of the task.Evidence suggests that animals that acquired the task at the breeding facility subsequently performed better both in early home enclosure sessions upon arrival at the research facility, and also in neuroscience lab sessions. Therefore it may be possible to use the automated system at the breeding facility to pre-screen animals for suitability for behavioural neuroscience research, and to use both the breeding and research facility systems to facilitate acquisition and transference of learning. Thus the use of automated systems could potentially reduce animal numbers required for studies, refine training protocols and minimise requirements for food/fluid control. This project was supported by the NC3Rs.

PB5 A new spontaneous mutation in the Lyst gene

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Analysis of spontaneous mutations can be investigated in a relatively straightforward manner using laboratory animals. Controlled laboratory environments and genetic homogeneity mean that new genetic changes can be quickly and precisely identified. Genes responsible for coat color also influence hematopoiesis, gonad formation, development and functioning of the nervous system and sensory organs. Additionally, these mutations often correlate with tumorigenesis and metabolic disorders.
Material and methodsNew spontaneous mutations associated with a diluted coat color phenotype have been reported in the CBA/W inbred mouse strain. Dilute coat color mice started to be bred as a substrain in 2008. Phenotypic changes were identified to a similar extent in both sexes. Mice were monitored daily for signs of chronic progressive diseases and evaluated for inactivity, weight loss, respiratory disorders, poor grooming and visible tumors. Detailed necropsies were conducted and organs were taken to carry out detailed pathological studies. Mutated animals were crossed with C57BL/10W mice in order to map the mutation by QTL analysis and find the mutation by sequencing these animals cDNA. Experimental procedures performed on mice were approved by the Local Ethical Commission for Animal Experimentation. Results and discussion The typical changes observed in CBA/W mutated mice were: slightly diluted color of the coat and skin (pallor skin coloration was visible in 3 day old mice) and smaller than in initial strain, and almond-shaped eyes. No significant differences for specific blood parameters were found, although a tendency for increased leukocyte and granulocyte counts were observed. The frequency of neoplasms detected post-mortem in mutated animals was 38,2% (compared to 55% in wild type strains). The spectrum of neoplasms was similar to the CBA/W strain and contained tumors of the liver, lungs and uterus. In mutated males (10 cases of 18) paraphimosis was observed. Mean life-span of mice homozygous for the detected mutation was significantly shorter than for non-mutated mice (586 vs. 910 days, respectively). In the hair, skin and eyes of the mutated mice irregular, giant granules of pigment were detected, and we confirmed the presence of cytoplasmic giant granules in granulocytes. Genetic mapping was carried out by crossing the mutated mice with C57BL/10W mice. Crossing confirmed that the observed mutation is inherited as an autosomal recessive trait. Genetic mapping revealed the genetic locus, linked to the altered phenotype, in the proximal part of Chr13, and Lyst was identified as the candidate gene. Sequencing analysis of the Lyst coding sequence revealed a C3181T nonsense substitution (Gln/STOP) in codon 1061. Thus, in mutant mice the LYST protein is truncated by approximately one third of its normal length. We suggested that this mutation may represent a potentially useful animal model of the mild variant of the Chediak – Higashi syndrome (CHS).

PB6 Changes in mRNA expression of opioid and cannabinoid receptors among three different mice strains after intrarectal exposure to capsaicin

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Rodent models are commonly used to study hypersensitivity, contributing to our understanding of the mechanisms involved in the treatment and management of pain. Significant differences have been reported when nociceptive behaviour is compared among strains of mice, fact that could explain the discrepancies when nociceptive studies are compared. The objective of our study was to compare basal and after capsaicin stimuli the expression of opioid and cannabinoid receptors in several mice strains
Material and methods: Two inbred mouse strains (C57BL/6J and CBA/J) and one outbred mouse strain (CD-1) were used (Charles River, France) in this study. Female (25-35 gr) mice underwent a laparotomy under general anaesthesia. 24 hours later, one group of animals was exposed to intrarectal saline (50uL) solution while another group was exposed to capsaicin (0.1%, 50ul). After 20 minutes, animals were euthanized and samples of colon and DRGs (T10-T13 and L6-S2) were collected. Changes in mRNA expression of mu (MOR) and delta (DOR) opioid receptors, and cannabinoid 1 (CB1) and 2 (CB2) receptors were determined. Results: No differences were observed between animals of the three different strains in basal conditions neither in colon nor in DRGs. Nevertheless, after capsaicin exposition, in CD-1 mice an increase in DOR, MOR, CB1 and CB2 receptors expression was observed, while expression remained unaltered in CBA/J and C57BL6/J. Similar results were found for CB2 receptor in thoracic DRGs. In contrast, in lumbosacral DRGs, an increase of CB1 expression was observed only in C57BL6/J. Conclusions: Although preliminary,

these results indicate that nociceptive receptor expression response may vary depending on mice strain. Therefore, further studies are needed to characterize the expression of these receptors according to mice strain, because differences at molecular level could explain the differences observed in behavioural studies.

PB7 Application of Microscopic Examination in Animal Models of Intraperitoneal Inoculating the Echinococcosis

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Xinjiang is a high prevalence area of hydatid disease, hydatid disease has become the constraints of local economic development and social stability of public health problems. This study through animal models of hydatid disease in microscopy observation, the establishment of animal model of rapid diagnostic methods for exploring the mechanism of hydatid disease animal model of infection, pathological changes and treatment effect evaluation provided a test platform.
Material Clean gray hamster (Cricetulus Migratorus) (60d), male and female, weight 35-45g, a total of 48 C. Migratorius. Methods Multilocular echinococcus in a dose of 2000 protoscolex/ml were intraperitoneally inoculated in the C. Migratorus. Using a simple visual observation and microscopic examination method, the growth of intraperioneal cyst, cyst fluid and protoscoleces was observed in the 10d,15d, 18d, 22d, 39d and 60d after inoculation. The growth of C. Migratorus peritoneal cyst, cyst fluid and protoscoleces; set up the blank control group (n = 8). Blank group and 60d group synchronization treatment, animals were necropsied, intraperitoneal cyst, weighing cysts and animal weight, macroscopic observation, measurement, counting and microscopic examination. Results Protoscoles were found in the 18d, and cysts became big and many in the 15d,18d and 22d. There was protoscoles blastemal growth in the 39d. Different degree of protoscolex was developed in the 60d. With the extension of the inoculation time, the cyst weight increased. 10d group,only 1 male Cricetulus migratorius autopsy found no cysts, the infection rate was 85.7% (6/7); 15d group, 2 male rats found no cysts, the infection rate was 50%, 4 females all cysts, female infection rate is higher than the male. The total infection rate was 75% (6/8); infection 18d, 22d, 39d and 60d group of female and male Cricetulus migratorius intraperitoneal were E. M, the infection rate of 100%. The blank control group in the same period in 60d fed hamsters at necropsy, there was no intra-abdominal vesicle like structure. Conclusion This experiment provides a simple and rapid method for the observation of the preparation of hydatid disease animal model. By the appearance of the naked eye observation of hydatid cysts growth, microscope examination found in protoscolex of growth, perhaps as a rapid detection of hydatid disease animal model method, but more important is can be observed in time protoscolex of growth development status, infection and the infection rate, lay the effective detection method for establishment of grey hamster multilocular hydatid animal model.

PB8 Establishment on enterocoelia animal model of Echinococcus muhilocularis in grey hamster (Cricetulus migratorius)

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The aim was to make animal model of Echinococcus muhilocularis in grey hamster, and observe growth rhythm of E. M in enterocoelia of the hamster. The experimental results show that there are already mature protoscoleces of cyst fluid in the hamsters abdominal at 18d, and the infection rate can reach to 100%.
Abstract: Materials Nighty two of 3-month-old gray hamsters (C. migratorius) (46 males and 46 females, body weight 35-45g) were used. Methods Ten gray hamsters were inoculated with Echinococcus muhilocularis (E. M) at doses of 100 500 and 2000. Animal were euthanized at 100 days after inoculation and the infection rate was observed. In addition, 62 gray hamsters were injected intraperitoneally at dose of 2000 E. M, and euthanized at 10, 15, 18, 22, 39, 60, 80, 100 days after infection. The cystica weight, serum antibody titers, ratio of cystica weight to body weight and the growth rhythm of E. M were evaluated. Results The cystica coefficients were 4.7%, 7.8% and 14.8% at 100 days after infection for the 100, 500 and 2000 dose groups, respectively. The cystica coefficient of the 2000 dose group was 0.36%, 2.83%, 8.22%, 15.37%, 17.6% and 13.87% after infection at 18, 22, 39, 60, 80 and 100 days, respectively. The antibody positive rate of the hamsters was 14%, 24%, 76%, 78%, 90%, 100% and 100% after infection at 15, 18, 22, 39, 60, 80 and 100 days, respectively. The serum antibody titer of the hamsters was 0.2, 0.4, 2.2, 3.4, 4.4, 5.8 and 6.4 after infection at 15, 18, 22, 39, 60, 80 and 100 days, respectively.Discussion The aim was to establish an animal model of Echinococcus muhilocularis in grey hamster and observe the growth rhythm of E. M in enterocoelia of the hamster. The experimental results show that there are already mature protoscoleces of cyst fluid in the hamsters abdominal at 18 days and the infection rate can reach to 100%. An infection rate of 100% in different doses for the hamsters inoculated with E. M after 100 days implies that the abdominal environment of the animals was suitable for the growth and development of E. M. The cyst coefficients in the hamsters infected with high doses was two times higher than those with the low dose. The infections with the high dose were suitable for a short cycle of animal experiments whereas the low dose was suitable for animal experiment of longer cycles or the conservation of strains. In addition, the cysts coefficient in females was significantly larger than that in the males, regardless of infection time or dose. This result indicates that the female is a more suitable model for E. M than the male. Furthermore, the increase of antibody positive rate and antibody titers with cysts coefficients can be used as an important indicator for evaluation of the animal model of E. M.

PB9 Research on reproductive performance of Meriones meridianus indoor

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Meriones meridianus belongs to Rodentia, Cricetidae, Gerbillinae, Meriones small rodents, distributed in Eurasia in the middle of the desert, semi desert region, In this paper, Xinjiang experimental animal research center from 1996 to 2002 Meriones meridianus Yip's subspecies breeding and development of statistics and analysis, in order to provide basic data for the rats of experimental animals and biological research.

Objective: The purpose of this study is to investigate the reproduction characteristics of Meriones meridianus in laboratory. Methods: According to the information of Meriones meridianus from 1996 to 2002 in Center for Laboratory Animal of Xinjiang, the sexual maturity period of male and female animals, and gestation period of female animals, and the litter size, weaning rate, survival rate, sex ratio per month and different fetal times were statistically analyzed. Results: Sexual maturity period of male and female animals was 109.3±21.0 days and 106.3±21.7 days, and gestation period of female animals is 21.3±1.4 days. The litter size, weaning rate of different fetal times showed no significant difference compared to the first to the seventh litter (P> 0.05), and the survival rate of fourth litter was statistically higher than that of the first litter (P < 0.05). The sex ratio of the first litter indicated no significant difference compared to the other litters (P > 0.05), and the average proportion of male and female is 1.4:1.0. The period of stopping breedfrom September to November, almost not breed, the diffrences between the litter size of other each month were not statistically significant (P>0.05), the weaning rate and survival rate per month suggested a significant difference(P<0.05) between the part of the month. Conclusion: Compared with the background data of wild Meriones meridianus, the laboratory reproduction of Meriones meridianus was difference, the season of breeding advanced to December, and the number of reproduction increased to 1 or 2 litters. Our results provided a reference for laboratory animalization of Meriones meridianus.

PB10 Diabetic cat as a Model for Human Diabetic Nephropathy

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Type 2 Diabetes Mellitus (DM) is the main cause chronic kidney disease (CKD) in humans. Feline DM shares similar clinical presentation with human type 2 DM, and represents a spontaneous model for this disease, but the effects of DM on the risk of chronic kidney disease have yet to be determined. Our aim was to evaluate the epidemiology and clinical presentations of feline DM and CKD in a population from Gran Canaria, Spain A retrospective study of clinical records from January 2014 to December 2015 was done in our Veterinary Teaching Hospital. Cats with DM and CKD were identified, based on clinical and biochemical data. DM was defined by clinical signs such as polyuria/polydipsia (PU/PD), weight loss, hyporexia, or vomiting, and hyperglycemia with blood glucose levels higher than 250mg/dL. The diagnosis of CKD was based on serum creatinine levels >1.6 mg/dL along with compatible medical history and clinical findings. Prevalence was calculated as the number of diagnosed cases divided by the total number of cats attending the hospital during the study period.Nine cats with DM were identified in a population of 1080 cats. (Mean prevalence 0.8%). Mean age at diagnosis was 9.4 years [4-15]. Eight were male, six non-sterilised and two sterilised. The only female was sterilised. Three breeds were represented among the diabetic cats: five European short-hair, two Persian, and two Siamese. Thirty-seven cats with CKD were identified. (Estimated prevalence 3.4%). Mean age at diagnosis was 8.7 years [1-17]. Twenty were males (four sterilised), and 17 were females (three sterilised). By breed, there were 26 European short-hair, six Persian, two Siamese, one Turkish Angora, one Maine Coon. Only one cat from the whole population was diabetic and also diagnosed for CKD ten years after the onset of DM. Based on these data, prevalence and breed distribution for DM and CKD did not differ from previous studies, which have also reported greater prevalence rates among European shorthair cats. Sex distribution was also similar to other populations for both CKD and DM, where males are at higher risk for DM presentation. Interestingly, from all studied cats, only one was diagnosed for both conditions. Although further studies are necessary to clarify the putative role of DM on feline CKD, these findings suggest that the relationship between DM and CKD is substantially different in cats and humans, being the first less prone to develop diabetic nephropathy.

PB11 Reproductive characteristics of an outbred colony of Peromyscus yucatanicus (Rodentia: Cricetidae): Viable model for Leishmania (Leishmania) mexicana studies

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In the Yucatán Peninsula of Mexico, Localized Cutaneous Leishmaniasis (LCL) is a wild zoonosis. The Yucatan deer mouse is a reservoir of L. (L.) mexicana. Studying immune response of a resistant host is primordial. With the recent DNA identification and cloning of Peromyscus' cytokines, this mouse is now an experimental model to study this parasite. The aim of the present research was to study the reproductive characteristics of Yucatán deer mice to enhance their environment and quality of life.
The breeding colony was derived from P. yucatanicus captured in a medium-size forest in the state of Campeche, Mexico. The overall successful pairing rate of 48.5% (N=270). All P. yucatanicus constructed a nest and pairs might share it or preferred separated nests however, nest building seemed a good indicator of reproductive potential and should be further investigated. This deer mouse reproduced all year long. Wild-born progenitors reproduced better than captive ones. Light fights resulted in tail biting however, mortal fights occurred. The most peaceful and successful pairings were between females of the same age as males. The best pup production with survival to over 6-months old was by females over two years old. The relative difference between the female and male weights was not related to either the success rate or fight between progenitors. However, the females that were heavier than their mate reproduced faster and took better care of their young than lighter females. In the wild, some P. yucatanicus pairs maintained the same territory for more than two years. Thus, the hypothesis that females would be more successful if paired repeatedly with the same male was tested. When females were paired monogamously, the interval between pairing and birth was shorter. Males paired with different females seemed more aggressive. Females isolated for more than 100 days between pairings had a lower success rate, more fights and a longer interval between pairing and birth of a litter. Fighting was further reduced and casualty ended with the use of cardboard tubes and boxes and nutritional enrichment. This study permitted to enhance the reproductive environment of Peromyscus and establish a sound outbred colony for the study of L. (L.) mexicana.

PB12 Evaluation of environmental pollution by recombinant adeno-associated virus (rAAV) upon intrathecal administration in swine: A Pilot Study.

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rAAV vectors are acknowledged as a promising treatment for congenital disorders as shown by the increasing literature in both animal models and humans. Proper management of genetically modified micro-organisms (GMMs) is extremely important for the safety of operators and environment, as sanctioned by Italian Legislative Decree n° 206/2001 and by European directive 2009/41/EC. Aim of the work is to evaluate the environmental pollution by rAAV in pigs within our experimental facility.
Materials & Methods Animals. Ten 30 days old WT (wild type) piglets were transferred from a local piggery to the swine facility of the Department of Veterinary Medical Sciences of Bologna and housed in a Class II biosafety level room according to the Italian and European laws. Before the arrival the room was sanitized using a hot pressure washer and Virkon® S. Pigs were divided in two groups: 5 in Box 1 and 5 in Box 2. According to the approved protocols, animals were administered with rAAV either via Cisterna magna (Romagnoli et al., 2014) or using a lumbar spinal catheter (Lambertini et al., 2015).Sampling. Swabs (saliva-SS; environment-ES) were collected starting from the day before the treatment at fixed time points. SS were collected individually on each animal on the day of injection (t0), 7 (t7), 15 (t15) and 30 (t30) days after. ES were collected rubbing the swab in different spots (slatted floor, box dividers, manger) of the crate everyday from the day before (t -1) to 7 days after (from t-1 to t 7), then 15 (t15) and 30 (t30) days after. The same sampling procedure was performed on additional non-treated animals and in their crate (Box 4), adjacent to the treated animals in the same room. Analyses. Swabs, previously stored at -20°C, were eluted using 570ul of Dulbecco's phosphate-buffered saline (DPBS) and vortexed for 30 seconds. 420ul of supernatant were collected and stored at -20°C until further analyses. DNA was extracted using the commercially available QIAamp® DNA Stool Handbook (Qiagen®) kit following the manufacturer's instructions and analyzed using a PCR protocol standardized in our laboratories [2]. Results Preliminary data show the kinetics of rAAV pollution in the environment, giving insights on any possible viral contamination both spatially and temporally. The results proved the technique to be sensitive and reliable. DNA extraction was easily performed using the standard manufacturer's protocol. Discussion and Conclusion The present work provides important data regarding the biology of the rAAV and the safety of their use in an important biomedical model as the swine. The evaluation of non treated animal housed next to treated one represents an important point to achieve better management of experimental facilities involved with gene therapy. In conclusion, this study, although preliminary, might help in the development of new safety standards for GMMs-approved facilities.

PB13 The immunological function of mesenteric lymph nodes in diet induced obesity.

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Obesity emerged into an epidemic that is associated with cardiovascular disease, insulin resistance and probably intestinal inflammation. It leads to a systemic inflammation, but it is unknown whether diet induced obesity would selectively influence the severity of Th1-based allergic contact dermatitis. The mesenteric lymph nodes (mLN) are important for immune responses. However, the immunological the function of the mLN in systemic inflammation after diet induced obesity has to be investigated.
Materials and Methods: Male C57BL/6N mice were fed a high fat diet containing 60% of calories from fat or a control low fat (10%) diet for 16-20 weeks. During this time body weight was determined and glucose tolerance was measured in the beginning and the end of this study. Afterwards mLN were removed and analyzed by real time PCR and flow cytometry. In addition to this, gut lavage samples were used for an antigen specific ELISA. Nine weeks after feeding the animals were sensitized on their depilated abdominal skin against the haptens 2,4-dinitrofluorobenzene (DNFB). Allergic dermatitis was challenged by topical application of the haptens on the ear skin after 16 weeks and ear thickness was measured 24 hours later. Results: Our results displayed that in obese mice the cell subset composition in mLN changes within 20 weeks. Analysis of the inflammatory response revealed that anti-inflammatory cytokines were reduced whereas pro-inflammatory cytokines such as IL-2 and IL-6 and antigen specific immunoglobulines were increased. Although DNFB treatment as a model of allergic contact dermatitis showed no significant increased ear swelling in obese mice, immune cells in the mLN were strongly activated. This was characterized by increased homing on all lymphocyte populations.Discussion and Conclusions: In summary, obesity resulted in mobilization of inflammatory cell subsets, an elevated level of pro-inflammatory cytokines and activated immune cells into mLN. These findings indicate a pivotal function of the mLN activating immune cells after died induced obesity and provide new insights into the immunological mechanisms of obesity related systemic inflammation.

PC1 Plasma concentrations of buprenorphine following a single subcutaneous administration of a sustained release formulation of buprenorphine in sheep.

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The goal of the present study was to measure the plasma concentrations of buprenorphine and its main metabolite, norbuprenorphine, following a single administration of a subcutaneous SR buprenorphine formulation in sheep. To our knowledge, no pharmacokinetic data relating to the administration of such formulation to sheep is presently available.
Materials and Methods: Twelve adult female sheep (6 Dorset and 6 Suffolk, 12 months of age) were used for this project and were divided into two experimental groups (n=6/group comprising 3 Dorset and 3 Suffolk sheep). SR buprenorphine was administered subcutaneously in the scapular region at a concentration of 0.1mg/kg for group 1 and of 0.05mg/kg for group 2. Following blood collections at selected time points, plasma concentrations of buprenorphine was performed by tandem liquid chromatograph - mass spectrometry. Results: Mean buprenorphine concentration was above 0.1ng/mL at 48h up to 192h post injection for group 1 and it was above 0.1ng/mL at 48h up to 72h post injection for group 2. Conclusions: A long lasting potential analgesic plasma level of buprenorphine is attained following a single subcutaneous injection of 0.1mg/kg SR buprenorphine in sheep. However, the effective analgesic plasma threshold still needs to be determined in sheep.

PC2 Anesthetic effects of isoflurane with carrier gas of oxygen and air mixture in mice

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Objective: Isoflurane has been used in laboratory animals including mice. When air is used with carrier gas of 3% isoflurane, mice occasionally have respiratory depression and decreased oxygen (O2)-saturation. When we use isoflurane anesthesia, we have to choose air or 100% O2 as the carrier gas. Recently, a unit that can add O2 to air (an O2-adding unit) has come on the market. In this study, we investigated the efficacy of this O2-adding unit for isoflurane anesthesia by measuring vital signs.
Methods: Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University. Three percent of isoflurane anesthesia was used on male ICR mice. Flow rates were 300 ml and 500 ml/min. For both flow rates, we added 0, 100, 200 and 300 ml/min of O2 to air for carrier gas. Six mice were used for each experimental group. Vital signs such as O2-saturation, heart rate, and respiratory rate were measured using a plus oximeter. We took measurements before and after anesthesia at 5 minutes (min), 10 min and then every 10 min until 60 min. We also measured a non-anesthesia group for vital signs. Results and Discussion: The groups using only air for carrier gas showed decreased O2-saturation levels compared to other O2-adding groups. There were no significant differences of O2-saturation levels between each group no matter how much O2 was added to the air. Anesthesia groups showed lower heart rates compared with the non-anesthesia group at 5 and 10 min after anesthesia. Anesthesia groups had a decreased respiratory rate compared with the non-anesthesia group from 5 to 20 min at flow rate of 300 ml/m. and from 5 to 40 min at flow rate of 500 ml/min. Lumb, A. B. et al. reported the perioperative oxygen toxicity which indicated that oxygen molecules can produce reactive oxygen species, which damage cells by reacting with the crucial molecular components (Anesthesiology Clin., 2012). These results indicate that the O2-adding unit is useful for inhalational anesthesia in laboratory animals.

PC3 Modified ice block as an alternative type of occupational enrichment for adult Göttingen minipigs

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A proper enrichment technique is included in the Directive [2010]/[63]/EU as a requirement when housing animals used for scientific purposes. As minipigs grow older, they tend to lose interest in most toys that used to interest them when they were younger. Therefore, enrichment needs to be adapted to the age of the minipig in order for it to reach its full potential. A project investigating the effect of modified ice blocks as enrichment was carried out in different age groups of Göttingen minipigs.
Materials and methods: Some of the main characteristic known to attract pigs' attention are that objects are ingestible, manipulative, deformable and chewable. The ice cubes were designed accordingly to this, e.g. irradiated straw sticking out of the ice cube can be chewed on, the ice itself is destructible/deformable and the food inside is ingestible. A long metal chain was put inside the bucket of water before freezing. By doing so, we could attach the large ice cube to the sides of the pens, preventing the ice cube from being pushed down into the manure section. Results: The ice cube attracted attention from all pigs in the tested pens in the section housing the adult minipigs. The lasting effect of the ice cube naturally depends on the number and size of pigs in the different pens. In pens housing 10 minipigs of an age around 9-10 months, the ice cube lasted for 1.5-2 hours under constant interest from most of the minipigs. When pigs play with the ice cube, the attached metal chain rattles. This sound and movement attract the pigs to the chain and gives a chance for pigs of lower hierarchal status to play as well. Discussion and conclusion: The size of the ice block makes it most appropriate for larger minipigs. Smaller minipigs around the age of 2-4 months showed either no interest in the ice block or were scared about it, possibly due to its size. For young minipigs smaller modified ice cubes. The ice block proved to be a good occupational enrichment method for the adult minipigs. This combined with the fact that the ice block is cheap to produce and easy to adapt, makes it a suitable alternative to other types of occupational enrichments for adult Göttingen minipigs.

PC4 Welfare assessment of genetically altered mice in Phenomin, a French multi-site phenogenomics infrastructure.

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Since adoption of Directive 2010/063 EU, many specific provisions had to be implemented to improve protection and welfare of animals used for scientific purposes. Among them, welfare assessment of newly created genetically altered (GA) mice has to be considered, as their use in research is increasing. Our Infrastructure Phenomin is a major player of phenogenomics with GA mice. We present here how we've implemented welfare assessment in our facilities.
PHENOMIN is a multi-site research infrastructure of excellence for translational research and functional genomics. It provides a comprehensive set of specialized services to academic and industrial users by combining the capacity of generating GA mice on a large scale with a high-throughput and comprehensive phenotypic analysis of the animals. The international Mouse Phenotyping Consortium (IMPC) is one the biggest scientific effort to understand mammalian gene function with GA mice where Phenomin is also actively involved through the generation of 235 GA lines. 3 French Institutes are involved in this project. After the French transposition of the European Directive in 2013, a Phenomin working group, mostly composed of animal facility managers and veterinarians, has been set up in order to establish a common process of welfare assessment on our new mouse lines.For that, we have followed the recommendations from the European Working Group on severity assessment specifying that welfare assessment should be performed:- When the line is established (From F2 onwards)- At 3 key time-points (after birth, around weaning and following sexual maturity)- On 7 animals per gender and per genotype, from 2 different litters as a minimum. We have created a first tool for animal caretakers to record their daily observations easily in an Excel spreadsheet with some Macros. A set of criteria dedicated to neonates and to grown-up animals from high level categories such as appearance, behavior, clinical signs, and relative size has been used. Mendelian ratios and fertility are also followed. A second tool, which is a scoring sheet, is used to interpret those observations, score the phenotype and compile data in a kind of passport, a document which is to follow mice when the lines are distributed worldwide. This passport will ensure that specific information related to animal welfare is accessible to whoever will care for these lines. The welfare assessment framework provides improvement welfare by minimizing the potential for pain, suffering and distress. The other benefits include also (i) an improved communication with a real connection between animal caretakers and researchers, (ii) new skills acquired for our animal caretakers as this welfare assessment was seen as a first step in our mouse phenotyping pipeline giving then, (iii) a comprehensive scientific information on GA mice.

PC5 Establishment of humane endpoints in rat model of mammary cancer

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Animal models are important tools to study the physiopathology and to develop new therapeutic strategies in several diseases, namely in cancer. Researchers are responsible for maintaining the animals' welfare. For this, they should establish adequate humane endpoints to each model of disease. The present work intended to define the adequate humane endpoints in an assay of mammary cancer using a rat model.
In this protocol, twelve female Sprague-Dawley rats were randomly divided into two experimental groups: MNU (n=10) and control (n=2). Animals were housed under controlled conditions of temperature, relative humidity, ventilation and light/dark cycle. At seven weeks of age, animals from MNU group received an intraperitoneal injection of the carcinogen agent N-methyl-N-nitrosourea (MNU) at a dose of 50 mg/kg. Animals from control group received an injection of saline. The following humane endpoints were proposed before the beginning of the experimental protocol: body condition; body weight; posture; coat and grooming; mucosal; eyes, ears and whiskers; mental status; response to external stimuli; hydration status; respiratory and heart rate; body temperature; location, macroscopic evaluation and mammary tumors' burden (tumors had a dimension higher than 35 mm). Animals were monitored twice a day. They were humanely sacrificed 18 weeks after the MNU administration. All procedures followed the European Directive 2010/63/EU and National Decree-Law 113/2013. As expected, no animals from control group developed mammary tumors. Six animals from MNU group developed mammary tumors (incidence of 60%). From these, five animals developed at least one mammary tumor higher than 35 mm. The remaining parameters were normal and the animals maintained healthy during the course experimental protocol. Once the animals did not exhibit any signs of pain or distress that implied their sacrifice, they were only sacrificed 18 weeks after the MNU administration, as previously planned in the beginning of the experiment. The proposed humane endpoints were considered adequate to monitor the animals' health status during a protocol of mammary carcinogenesis. Looking to these results, it was also possible to conclude that the alteration in only one humane endpoint do not imply the animals' sacrifice. The endpoints should be evaluated together, in order to define the most adequate time in which the animals should be humanely sacrificed.

PC6 Effectiveness and resistance of a new toy introduced in unisex groups or pairs of cynomolgus macaques housed in standard or depleted environments

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Primatology centers house non-human primates (NHP) in various environments. More and more effort is made to implement an environmental enrichment program to enhance the animals' welfare in captivity. One of the most used non-edible enrichment for NHP is the "toy". It has to be handleable, non-toxic, and resistant. Above all, its effectiveness has to be checked. . The effectiveness and resistance of a "Zogoflex" range toy (Plexx) has been tested for the first time in NHP at Silabe (France).
One "Bumi" toy has been introduced in 4 different conditions in cynomolgus macaques groups: one group of young (≤ 3yo) females and one group of adult (> 6yo) males housed in a "standard" environment (social group of about ten individuals in an enclosure with outside access); and one pair of young females and one of adult males housed in a "depleted" environment (enclosure without outside access). Behaviors have been video-re-

corded. Data have been collected using a toy-focal sampling method applied to four continuous observational periods of 2 hours distributed over the 48 hours after the introduction of the toy. An interaction behavior with the toy has been defined whenever at least one individual touched it with its own body. Moreover, the localization of the subject interacting with the toy has been specified at each statement. Finally, toys were left in enclosures were visually checked every week for resistance assessment. Data showed that the 2 groups of animals housed in a standard environment interact with the toy during 92,9% of time for the young females and 80,4% for the adult males. On the other hand, the pairs of animals housed in the depleted environment spent 0,3% (young females) and 6,3% (adult males) of time interacting with the toy. Looking at the location of the animals, mean percentage revealed that macaques are more on an upper level while interacting with the toy than on the floor (71,3% versus 28,7%). Regarding the resistance, one out of four distributed toys has been seriously damaged within only two weeks in the group of adult males. It seems that this kind of non-edible enrichment shows few attractiveness for animals housed in a depleted environment in contrary to those housed in a standard one, whatever their age or their gender. This phenomenon could be explained by a stronger competition context in a group of about ten individuals than in a pair (Ballesta 2014). In general, we assume that animals housed in a group in an enclosure with outside access are better stimulated by what is happening in their home cage and therefore pay more attention to a new valuable resource added inside. On the other hand, we supposed that animals housed by pair in an enclosure without outside access are more worried about what is happening next to their home cage than inside. Regarding the general properties of this new toy's range, resistance and flexibility are satisfactory and suitable for a use in NHP, except for animals with big canines.

PC7 Could body temperature, food and water consumption be used as humane endpoints in urinary bladder cancer studies in rats?

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The application of appropriate and objective humane endpoints is crucial when refining in vivo cancer research. However, defining humane endpoints in cancer research can be challenging (1). Body temperature, food and water consumption are considered valuable biomarkers to monitor animals during cancer studies (2). We investigated whether these variables could be used as humane endpoints for urinary bladder cancer studies in rats.
Wistar rats (n=20) with four weeks of age were housed in polycarbonate cages (1264C Eurostandard Type IV, Tecniplast, Buguggiate, Italy) with corncob for bedding, undercontrolled conditions. Over the course of 20 weeks, animals received N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) at a concentration of 0.05% in drinking water (test group) or only tap water (control group) along the experimental protocol. After this period, animals were maintained with simple water until the end of the experiment. The animals were daily observed and body weight, food and drink consumption and rectal temperature, were measured weekly. Thirty-five weeks after the start of the experimental protocol, animals were sacrificed by intraperitoneal administration of sodium pentobarbital followed by exsanguination by cardiac puncture. The statistical analysis was performed by SPSS®. The differences were considered statistically significant at p0.05). The final drink intake was higher in BBN group, however the difference was not statistically significant (p>0.05). No differences were observed in rectal temperature between groups (p>0.05). Measure of body temperature, food and water consumption are easy to assess, since no specialized skills or equipment are required. In this study only the water consumption was higher in the BBN group, thus suggesting that drink could be probably a useful parameter to be analyzed. However, more specific markers should be evaluated, such as the collection of urine. In a previous study performed by our team, collection of urine showed the presence of macroscopic hematuria. Also different parameters such as the Rat Grimace Scale, behavioral tests and collection of biological samples for molecular assessment may be applied to complement these data.

PC8 The Humane Endpoints website: for refinement of animal experiments

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According to the European Directive 2010/63, researchers are obliged to consider the 3Rs (replacement, reduction and refinement) when designing and performing procedures involving animals. To accomplish this, the latest information about 3Rs-methods has to be identified. Databases and informative websites can facilitate retrieval of specific 3Rs-related information for scientists, but also for the institutional Animal Welfare Bodies, project evaluators (DEC's) and the Competent Authorities.
These databases and websites save time-consuming searches, facilitate completeness and contribute to the 3Rs. To be successful, they should be easily found, accessed, managed and updated. In addition, relevant data should be easily retrieved. By its 3Rs database programme, the 3Rs-Centre Utrecht Life Sciences (ULS) facilitates the search for and implementation of 3Rs methods. One of the databases within this programme is the Humane Endpoints website (www. Humane-endpoints.info). This website provides information about humane endpoints and teaches to recognize humane endpoints in laboratory animals. Subsequently, further suffering of the animals can be prevented by removing the animals from the experiment (e.g. euthanizing the animals) or by applying analgesia. The website contributes to refinement: the prevention of unnecessary suffering in laboratory animals. The Humane Endpoints website includes a wealth of information on humane endpoints and related aspects and has an extensive database of videos and photographs of clinical indications. In addition, it provides an interactive educational component for training purposes and is therefore used in several laboratory animal sciences courses worldwide. The website is available in English, Dutch, French and Spanish. Later this year, a German version will be available. Furthermore, information about humane endpoints in fish used for laboratory purposes will be added to the already existing information on mice and rats.

PC10 Buprenorphine in rodents: more harm than good?

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In spite of numerous positive testimonies in literature of buprenorphine as an effective and safe opioid analgetic, Janssen Research & Development in Beerse, Belgium has decided to abandon the routine use of buprenorphine for per-operative analgesia in rodents after a series of incidents in rats and mice.
Many authors have already reported on the behavioral side-effects of buprenorphine, which include many different features: pica-behavior, stereotypic eating/biting, increased activity/excitation but also sedation. At Janssen Research & Development (Janssen R&D) in Beerse, Belgium we have been confronted with a series of incidents in rats and mice treated with buprenorphine pre-operatively (0.025 up to 0.05 mg/kg), that in the first hours after surgery showed compulsive biting on the stitches and wound clips. This resulted in in open wounds which was an unexpected complication not described in literature. These observations came on top of the known adverse effects of buprenorphine. Based on these findings and because of the availability of other (opioid) agents with adequate analgetic efficacy, that do not show these side effects, the Working Group on Experimental Surgery of Janssen R&D decided to abandon the routine use of buprenorphine for peri-operative analgesia in rodents.

PC11 A risk-based approach to reducing exposure of staff to anaesthetic gas.

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The risk of pollution by anesthetic gases in animal procedure rooms is examined and the methods to reduce exposure is explored with emphasis on the work health and safety aspect of personnel. As to prevention, measures for ensuring safe use and maintenance of devices used in the laboratories are indicated, as well as training procedures in effective use of inhalational anaesthetics adapted by the institution.
It is well known that there is potential occupational health hazard associated with exposure to anaesthetic pollution. Safe anaesthetic practices is very important in conjunction with installation of appropriate scavenging system to reduce risks and reduce personnel exposure to waste gases. Also imperative is that the use of systems in place must be correct and properly maintained. At the Children's Medical Research Institute, a review of institutional practices on use of rodent anaesthetic machines has resulted in increased safety awareness and improved working environment for personnel and that of the animal. We utilise the risk assessment matrix to identify the level of risk and help prioritise the implementation of risk controls. This presentation includes a discussion of modifications to the equipment that affect both the animal care staff and researchers working within the unit. It covers the procedures and documentation used via an online learning management system to record training and keep track of progress and reports. Users must complete a two-part training process for independent use of the anaesthetic machine. This involves reading and declaring compliance to the Standard Operating Procedure and completion of a practical training session that must be acknowledged by signing off on the training series. The training platform allows us to maintain a central information management database that captures work health and safety training and education records on an ongoing basis to act as a reference tool and enable easy retrieval of data during compliance inspections. The presentation also describes the importance of the trainer's continuing education ensuring up-to-date knowledge is relayed to staff members when conducting group training sessions and the value of feedback from users to help make their work with animals easier with quick reference tool guides on proper use of the equipment.

PC12 A novel home cage monitoring system for multiple-housed mice

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Many CNS disorders and neurodegenerative diseases are investigated using mouse models. Experimental designs involve placing mice into novel environments to undergo a battery of tests, often conducted for short periods in social isolation. However, human manifestations of such disorders are often progressive and have a large social element. Here we introduce an automated system to assess disease progression, animal welfare and investigate social behaviours of mice in their homecage environment.
The Home Cage Analysis (HCA) system, devised by Actual Analytics, Edinburgh, is built around a standard Individually Ventilated Cage (IVC) and comprises a radio-frequency identification (RFID) reader baseplate as well as an infrared camera and a computer. The cage sits on top of the baseplate, docked within its usual rack and infrared lighting above the cage enables continuous video capture. With this setup, we are able to record both activity data, via the tracking of the microchips, alongside videos which enables detailed analysis of behaviours of up to five mice within a cage. The current set up has been optimised to three mice in a cage.Using this system, we have been able to measure parameters such as locomotion, pair wise separation and time spent in isolation for each mouse in the home-cage environment. We have been able to get a unique insight into the light phase activity of mice and analyse differences in activities of various background strains over the light-dark cycle. Through machine learning, behaviours such as grooming, feeding and social interactions can be automatically annotated without experimenter intervention over extended periods of time. This has enabled the measurement and analysis of multiple parameters simultaneously under group housed conditions, making this a powerful tool in the identification of novel behaviours. The system has enabled us to monitor long term health issues and identify, at a much earlier stage, behaviours which may impact animal welfare. In addition, this information has proved vital for designing future experiments in line with strategies to reduce and refine animal experiments, as we are able to take into consideration previously unknown factors, such as the impact of housing mice with strong phenotypes with their wild type companions. In certain cases we have identified behaviours which impact the health of mice which may be missed during routine cage side assessment. The unique combination of video and spatial data provides a much richer set of features for analysis and can be achieved through a minimally invasive procedure. In addition the system provides a tool to identify earlier time points for humane intervention for models of neurodegeneration, especially where these have been difficult to detect from cage side assessments. The potential impact for this project on the future direction of welfare and behavioural testing is significant and far-reaching.

PC13 Effect of anaesthesia on cardiorespiratory parameters in healthy and parkinsonian Macaca fascicularis.

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Non-human primates are important models for the study of Parkinson's disease (PD). In human patients PD, is frequently associated with perioperative complications; anaesthetic concerns are focused on malfunctions that may lead to respiratory dysfunction, cardiac arrhythmias, orthostatic hypotension, and defective control in temperature regulation. We describe the effects of one anaesthetic protocol on cardiorespiratory parameters in 17 macaca fascicularis before and after chemically-induced PD.
Injectable anaesthesia was used to perform a brain positron emission tomography (PET-CT) in healthy macaca (PET0) and in the same animals 3 months after 1methyl-4phenyl-1,2,3,6tetrahydropyridine (MPTP) parkinson induction (PET1). Sedation was achieved by intramuscular administration of xylazine (0.5 mg/kg) and ketamine (5 mg/kg). An intravenous catheter was placed, anaesthesia was induced with xylazine (1 mg/kg) and ketamine (10 mg/kg) intravenously (IV) and, after orotracheal intubation, each animal was connected to a non-rebreathing respiratory system delivering oxygen 100%. The animal was positioned onto the CT gantry in prone position. Respiratory rate (RR), end-tidal carbon dioxide (EtCO2), heart rate (HR), electrocardiography, non-invasive blood pressure (BP), saturimetry (SpO2) and temperature were constantly monitored by Cardiocap II (Datex – Homeda). Data were analysed by ANOVA test. Mean anaesthesia time was about 120 minutes in both groups. No complications were observed: in PET1, 2 animals showed self-limited cardiac arrhythmia. At the end of the procedure, all animals recovered within 15 minutes with a slightly prolonged recovery time in PD animals. In PET1 mean HR was significantly higher and BP was significantly lower than in PET0 (HR: PET0 86.66 ± 3.91 bpm ± sd; PET1 117.21 ± 4.38 bpm ± sd. $p<0.001$. Mean BP: PET0 91.95 ±11.51; PET1 60.01 ± 8.65 mmHg ± sd. $p<0.001$). Although a significant reduction in mean RR was observed in PET1 (PET0 26.41 ± 1.22 bpm ± sd; PET1 21.04 ± 1.28 bpm ± sd. $p<0.001$), EtCO2 and SpO2 did not differ between groups. Temperature was similar in both groups. PD involves different systems and therefore comprises multiple physiological functions. In this study, PD influenced the cardiorespiratory response to the anaesthetic protocol used. The increased HR in parkinsonian animals may be a consequence of a reduction in BP as it is described in human patients affected by PD. In animal models MPTP induced an altered myocardial contractile function: we observed arrhythmia in 2 parkinsonian animals but xylazine may be responsible for this event. The reduced RR in PET1 did not influence EtCO2 nor SpO2: oxygen administration probably had a positive effect on oxygenation and compensated the respiratory depression in PD animals. The pathological condition and its systemic effects should be considered when anaesthetising parkinsonian animals. A careful monitoring of the cardiorespiratory function is highly recommended.

PC14 Prospective severity assessment in cephalopods: Results of an on-line survey of the COST Action (CephsInAction) FA1301 cephalopod research community

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The inclusion of an entire new Class of animals, "live cephalopods" in Directive 2010/63/EU necessitates the development of guidance on prospective severity classification of regulated procedures to be included in the project application. Consideration of factors affecting severity also informs protocol refinement and humane-end points. Here we report how the EU cephalopod community engaged with this challenge.
METHODS An on-line survey comprising short scenarios (<15 lines) based on a range of approaches appeared in published literature and containing sufficient key information to make an assessment of severity were written and reviewed by an "expert reference group" (RG) including experienced regulators. After review 50 scenarios were used including examples pertaining to below threshold, non-recovery, mild, moderate, severe and upper threshold. For each scenario an "unable to decide" option was included. Detailed notes about the principles of severity assessment were included based on Annexe VIII. A survey in English was then promoted through the COST Action FA1301 website (www. Cephsinaction.org) and members were invited to participate online. RESULTS The response rate was 33.5% (n=59) covering 15 countries. The overall "unable to decide" rate was relatively low (about 7%). Three "non-recovery" scenarios were identified with an average score of 69%. The average score for scenarios considered "above threshold" by the majority (+RG) that were incorrectly assessed as "subthreshold" was 12%. Scenarios involving behavioural studies had scores that bridged subthreshold and mild categories. A detailed analysis is presented. Respondents graded severity of scenarios across all categories and identified factors such as anaesthesia, surgery, drug injections etc and repetition of intervention as factors increasing severity. CONCLUSION The data is being used to develop objectively based guidelines for prospective assessment of severity in cephalopods and tabulated examples of procedures within each category (c.f. Hawkins et al., 2011 for fish). Overall the CephsInAction community was able with minimal information to identify the threshold for regulation of a procedure and factors affecting severity. It should not be overlooked that the majority of researchers in this area have not worked in a regulated environment and have had to adapt rapidly to regulatory requirements over the last 3 years (see Fiorito et al., 2015 for overview). The results also illustrate the utility of on-line survey of a community combined with quantitative analysis to provide an objective base for development of guidance.

PC15 Antinociceptive effects of voluntarily ingested buprenorphine in the hot-plate test in laboratory rats

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An adequate analgesic strategy is crucial to improve postoperative recovery and welfare of laboratory rodents, preferably supplied by minimally invasive methods. Previous studies have shown voluntary ingestion of buprenorphine effective as post-operative analgesia, but the effect in nociceptive tests have been debated. This study investigated the antinociceptive effects of buprenorphine administered orally in Nutella® compared to subcutaneous buprenorphine and alternative analgesic regimens.
Materials & methods: A hot-plate (52.5°C) was used to assess antinociceptive effects of analgesics during three trials in male Sprague Dawley rats: I) Investigation of subcutaneous (s. C.) buprenorphine (BUP) dose; effects assessed 60min post treatment (0.015; 0.05; 0.1; or 0.3 mg/kg BUP; vehicle/saline, n=10). II) Investigation of BUP dose in Nutella®; effects assessed at 30, 60, 120, 240, 360, 480 min post administration (0.1 mg/kg s. C. in saline; 0.5; 1.0 or 2.0 mg/kg BUP per oral (p.o.) in Nutella®; p.o. vehicle/Nutella®, n=10). III) Comparison with other analgesic treatments; effects tested at 30, 60, 120, & 240 min post treatment (BUP 0.1 mg/kg s. C.; BUP 1.0 mg/kg p.o. in Nutella®; carprofen 5.0 mg/kg s. C.; lidocaine 2.0mg intramuscularly (i. M.) in the mid-thigh; combination of BUP 0.1 mg/kg s. C., carprofen s. C. and lidocaine i. M.; saline/vehicle s. C., n=10). Results: S. C. administration of 0.1 mg/kg BUP was the most robust antinociceptive dose, inducing significantly increased response latency to the thermal stimulus with a rapid and strong onset from the first measurement from 30-240 mins post treatment. When 1.0 mg/kg BUP was administered in Nutella®, hot plate latency was significantly increased at 60 and 120 min, while 2.0 mg/kg showed effect at 120 mins post treatment, and the low dose of 0.5 mg/kg was ineffective. Carprofen and lidocaine failed to affect hot plate latency at any time point examined. However, the combined treatment appeared to have a synergistic effect when comparing with the individual analgesics alone, and at 30 min, the combined treatment was significantly elevated compared with all other groups (P<0.001). Discussion & conclusion: Oral dosing of buprenorphine brought a later onset of action and although antinociceptive effects were not as marked compared with s. C. administration, it was nonetheless regarded as an equally adequate method to provide postoperative analgesia when both first-pass metabolism and delayed onset of action is taken into consideration. Other studies suggests that administration of buprenorphine in Nutella® have benefits by prolonging the duration of high serum concentrations compared to s. C. administration (1). In the current study, a significant antinociceptive effect was evident at a dose of 1.0 mg/kg in Nutella®, but lower doses may be sufficient when treating post-operative pain, as other studies have reported improvements of post-operative recovery in rats at a dose of 0.4 mg/kg BUP in Nutella®(2).

PC16 An autonomous and automated device to assess concurrently several cognitive functions in non-human primates living in social group

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Discoveries are highly dependent on technological advances. In neurosciences, behavioural studies are crucial in that they can measure the visible part of cognitive processes. Research in cognition on non-human primate models has recently known a real disruption with the development of experimental devices allowing testing subjects maintained in their social group (Fagot and Paleressompoulle, 2009). This new approach quickly proved itself in various fields of social and non-social cognition.
Materials and methods: Here we propose an evolution of this approach through a new experimental device more adapted for a use in laboratory environment. This method permits to accurately measure cognitive abilities of monkeys housed in groups. It only uses operant conditioning, which excludes both deprivation and physical constraints. Subjects have a free and ad libitum access to the device thanks to an automated identification system. The cognitive paradigms are provided via a touch-screen interface, as in humans (Robbins et al., 1994). The original feature of this new device lies in that the difficulty of each task changes in real time according to individual performances involving that the full experimental design operates autonomously (i.e. testing and learning processes). In addition, several complex cognitive tasks can be learned and performed in parallel allowing the measurement of the joint evolution of several cognitive functions. Results: Here we present the results of two pilot studies that validate the efficiency of our mobile experimental device and our new experimental design. A group of macaque monkeys were trained and tested concurrently on three cognitive tasks highly used in research on aging and neurodegenerative diseases: the 5-Choice Serial Reaction Time task (attention), the Delayed Match to Sample task (short term memory) and the Self Ordered Spatial Search task (working memory) (e.g. Nagahara et al., 2010). The data show that the learning time of tasks was particularly short through this new approach without any human intervention. In addition, performance were very high and particularly stable over time despite that the subjects performed several tasks in parallel (e.g. Weed et al., 1999). Discussion and conclusions: We think that such experimental devices, easy to handle and allowing to collect a large amount of data while guaranteeing a good standardization of experimental protocols, could be valued i) in basic studies concerning the mechanisms underlying social and non social cognitive processes, as well as ii) in applied studies, e.g. pharmacological and biomedical researches. In addition, the procedures established are particularly in agreement with new ethical standards of animal experimentation.

PC17 A geometric morphometric analysis of craniofacial development in ketamine-exposed zebrafish embryos

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The use of zebrafish has been increasing, but this has not been accompanied by implications of anesthesia in this species research. Increasing experimental evidence has shown that exposure to ketamine, a widely used anesthetic, may be neurotoxic during specific developmental stages. Ketamine has been shown to also act independently from the NMDA receptor and to be teratogenic. Here, ketamine effects on the craniofacial development and its implications for risk assessment were evaluated.
Zebrafish embryos were exposed to previously reported concentrations of ketamine (0.2, 0.4 and 0.8 mg mL-1) in order to investigate stage-developmental toxicity of this pharmaceutical. During blastula (2.5 hours post fertilization- hpf), gastrula (5.5 hpf) and segmentation (10.5 hpf), embryos were exposed to ketamine for a period of 20 min and were allowed to grow until 144 hpf. At the last day of the experiment, zebrafish cartilage and bone evaluation were carried out using alcian blue and calcein staining protocols. Geometric morphometrics was used to quantify similarities and differences between head shapes of zebrafish larvae. Seven lateral-view landmarks and twenty-one ventral-view landmarks were used to define the cartilaginous morphology whereas twenty-nine ventral landmarks were defined to study bone morphology of the head. To visualize the position of morphotypes in environmental space, a scatter plot of principal component analysis (PCA) score values was used. Embryos exposed during blastula stage showed to be most sensitive to ketamine resulting in a more disperse variation on the head morphology, namely with the highest ketamine dose. A decreased head size, elongation of the head area and changes in head depth were observed in this group. Furthermore, the transformation grid reflected that landmarks that were most affected by ketamine treatment were positioned in the ceratohyal cartilage. Relatively to bone morphology, no significant changes were observed although some patterns might occur associated with ketamine exposure. Results showed that ketamine may notably influence head development resulting in craniofacial abnormalities and that phase-dependent responses could be successfully distinguished using a geometric morphometric approach. Together, it is concluded that the head shape analysis might be used as an explanatory tool to adequately assess the risk of ketamine to humans and wildlife. Furthermore this work shows that ketamine may induce potential non-wanted effects in zebrafish studies.

PC18 Development of user friendly and lower-cost approaches for automatic assessment to rodent movement

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The locomotor activity is an easily performed behavior measurement that gives important information. [1] Its assessment is traditionally made by simple observation, however, is subjective, tedious, time-consuming and requires practice. The results may also suffer from multiple errors as the observer's fatigue. [2],[3]Even though technology is advancing with the creation of complex video tracking or Radio Frequency Identification systems, there's a lack of methods to evaluate simple movement.
The automation of apparatus for behavioral studies with sensor's systems is available in the market, however the cost are still high. In this study, it's presented a software application, tested in a three-box connected by two tunnel apparatus, using a LC (load cell), IR (infrared) and LDR (light dependent resistors) sensors for animal detection. The light sensors (LDR and IR) were placed in the tubes' walls near to the boxes' entrances. The LC was placed in the left side of the apparatus. An application using LabVIEW2013 was developed to obtain the data from each group of sensors, filter and convert it to a digital classification. Tests were divided in two groups: Laboratorial tests and Experimental tests. In the first a small ball and a mouse replica were used to detect the response from each method to the passage of an object. The second one, used two locally breed, 13 week old NMRI mice. They were placed in the apparatus, one at a time, for around 5 minutes. In the Lab tests, the LC showed a bad response to dynamic movement. Thus, the method was excluded for the experimental tests, once the expected walking movement wouldn't give us useful information. Laboratory tests showed that the response of light sensors was good even though there were FN (false negatives) and FP (false positives). The LDR suffered from a huge influence of environmental conditions. Thus, we can see large signal variations through time, with FN and FP results. Those may not correspond to the passage of the animal, but a momentary change in the light conditions as the operator's interference. On the other hand IR correlated well with manual observations. The results of LDR sensors were also difficult to filter, convert properly and analyze manually. We can conclude that the infrared system is the more versatile for detection of passages. The IR method can be applied to various behavioral apparatus as radial maze, T-maze and Elevated plus maze, among others. The relationship between cost and benefit of the approaches presented is beneficial. However, there's still a long way in the search of an ideal method which allows a wide range of behavior detection with accuracy, reproducibility, flexibility and a competitive price

PC19 A refined protocol combining anesthesia and analgesia within the framework of rabies intracranial mouse inoculation.

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Although progressive replacement of rabies intracranial mouse inoculation has been applied using in vitro models (1), this technique is still inevitably adopted for the production of reference materials (2). Prevalent anaesthetic protocols do not guarantee proper analgesia and are responsible for side effects, ultimately leading to the animal's death. We aimed at refining a mouse anaesthetic protocol to be safely used in young mice by guaranteeing appropriate analgesia and a rapid recovery time.
178 CD1 3 weeks-old mice (13-14 g) of both sexes were obtained from an internal breeding facility in four different sessions. According to the available literature (3), the tested anesthetic protocol included the administration of 0.4 mg/kg buprenorfine by subcutaneous (SC) route. Thirty minutes later, ketamine+medetomidine 40/0.3 mg/kg were administered by the intraperitoneal (IP) route, and animals were placed on a warming pad (+37°C) immediately after the loss of righting reflex. After 5 minutes, the absence of nociceptive withdrawal reflex was confirmed and mice were inoculated intracranially with 30 ml of virus suspension by a 30 G needle inserted 2 mm under the skull. Animals were then placed again on the warming pad. After a15-minute interval, mice were injected by SC with atipamezole 1 mg/kg as reversal and recovered within few minutes.

Housing and procedures were in accordance with Directive 2010/63/EU and approved by the IZSVe's Ethics Committee. Anesthetic depth was considered satisfactory for the procedure in both females and males. Reversal was obtained after a few minutes and all animals recovered from anesthesia; no death was registered within 24 hours. Despite the effective application of in vitro techniques for both virus isolation and vaccine potency testing, the complete replacement of the intracranial mouse inoculation in the practice has still to be achieved. Although moderate to severe pain due to rabies clinical signs cannot be avoided since their onset represents the experimental endpoint, the severe discomfort due to the procedure itself should be avoided. Inhalation anaesthesia or common injectable anaesthetic protocols are mainly used for mice immobilisation during mild procedures. However, the lack of analgesia makes them unfit for intracranial inoculation. In addition, they may result impractical due to the long-lasting effect and the related hypothermia and hypoglycaemia. The proposed protocol is a safe and balanced alternative when a deep anaesthetic plane, rapid recovery and alleviation of post-operatory pain are requested in young laboratory mice. Indeed, the possibility to antagonise medetomidine and the rapid metabolism of ketamine allowed a fast recovery from anaesthesia in all animals, eventually preventing mortality. Considering the unavoidability of such a procedure, this protocol could largely alleviate discomfort for mice used in rabies laboratories.

PC20 Oral self-administration of paracetamol for pain relief after embryo-transfer surgery in laboratory mice.

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The aim of the present study was to determine whether voluntary paracetamol uptake with the drinking water is sufficient to cover post-operative analgesic requirements after embryo transfer (ET) into recipient female mice, i.e. foster mothers (1, 2). In addition, the impact of the analgesic on the outcome of the procedure was determined by comparing the offspring after embryo transfer with and without paracetamol treatment (3). Material & Methods: Water consumption was measured in foster mothers and in naïve female mice. Drinking water was either untreated or contained paracetamol in a dosage calculated to provide the mice with approx. 200 mg/kg paracetamol per day. In naïve mice, serum concentration of paracetamol was measured at 6, 11 and 24 h during which treated water was offered (n=8 per time point). Fifteen mice were used as foster mothers. After ascertaining pseudo-pregnancy, embryo recipients were randomly allocated either to the untreated (n=8) or the paracetamol treated group (n=7). Paracetamol containing water was provided at 6 h before ET and continued for two days. Each foster mother received 12 embryos; litters were closely monitored until weaning. Results: Paracetamol treated foster mothers delivered in average slightly more pups per litter than untreated animals. Therefore, the outcome of the ET was marginally better in the treated group compared to the untreated group. The body weight of the newborns was similar between both groups. No dead or injured pups were found at the time of birth and all pups were reared until weaning. Water consumption increased significantly if paracetamol was added to the drinking water with similar amounts consumed by naïve mice and foster mothers. Serum concentration of paracetamol reached a sufficient level at 6 h (time point where ET was performed) and increased further in the post-operative phase, i.e. at 11 h and 24 h. Conclusion: In summary, our results provided no evidence for adverse effects of paracetamol treatment on overall outcome of ET. Paracetamol in the drinking water was voluntarily consumed by female mice after surgery in an amount that allows the assumption of constant sufficient post-operative pain treatment. No relevant side-effects on the outcome of ET were detected thus supporting the administration of paracetamol in drinking water as a feasible and efficient method for providing analgesic treatment in mice during generation of genetically modified lines.

PC21 Long-acting transdermal fentanyl solution during perioperative analgesia in multi-species surgical research.

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Opioids are commonly considered as an important part of multi-modal postoperative analgesia on animal models for surgical research, but there are intrinsic limits including rapid clearance resulting in repeated subcutaneous injections. The objective of the study was to evaluate the efficiency of a long-acting transdermal fentanyl solution (LATFS) with quantification of the blood fentanyl concentration (BFC) at day 4 of follow-up on multi-species surgical models (primary endpoint).
1.3 mg/kg (half-dose) of a LATFS was topically applied, prior to surgery, in a single administration, onto the interscapular skin of four Landrace pigs. A free-opioid premedication and anesthesia was achieved. Blood samples were collected and evaluation of postoperative pain was achieved with a behavioral scale. In the same way, a 50µL drop (half-dose) of LATFS was applied in ten 300-grams Wistar rats prior to microsurgery experimentations. Quantification of fentanyl and its metabolite nor-fentanyl was performed with ultra high performance liquid chromatography coupled to mass spectrometry in plasma and total blood. At the end of follow-up, BFC were always above the therapeutic threshold (TT) (> 1 ng/mL) in both species and reached the TT at first blood sample. At day 4, mean BFC was 4±2.7 ng/mL in rat model. Maximal concentration was obtained at Hour 6 in pigs (27±9 ng/mL). The use of a single, simple and noninvasive administration of LATFS can sustain BFC above TT during at least 4 days and should be mostly used in surgical research.

PC22 How do you tell how long has a mouse been dead? Rigor mortis as a tool to estimate mice time of death (TOD) in animal house facilities.

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In rodent animal house facilities knowing how to estimate the time of death (TOD) is of the utmost importance since it may determine if collecting biological samples is still possible, or not. Rigor mortis is defined as the stiffening of muscles after death and it has been used as a tool to estimate the TOD. [1] Interestingly, is difficult to find accurate information regarding rigor mortis onset, development and resolution in mice.
Considering these difficulties, we decided to determine rigor mortis evolution in Mus musculus under stable environmental conditions. Materials and methodsAll animals were housed and maintained in accordance with Portuguese DL nº 113/2013 and kept under stable temperature (20 ± 1ºC, n=228) and relative humidity (26,9 ± 4,49%, n=228) conditions. Adult male and female C57BL/6 mice (n=11) with 4 ± 1 months of age and weighing 26 ± 2 gr were used in this study. Cardio-respiratory arrest (CRA) was induced by a single intraperitoneal administration of pentobarbital sodium (300 mg/Kg). Rigor was evaluated by flexing and extending the temporomandibular joint (TMJ), neck, front limbs (FL), hind limbs (HL) and tail and given a score from 0 to 4 according to the degree of rigidity. The eyes were observed as well and given a score according to the degree of corneal opacity and sinking. Measurements were made during the first five hours and between the 17th and 27th hour after CRA. ResultsThe results show that TMJ was completely blocked in first hour after CRA (0,88 ± 0,19 h), followed by the neck (1,33 ± 0,41 h) and front limbs (1,90 ± 0,74 h)

during the next hour. The tail (2,24 ± 0,75 h) and hind limbs (2,67 ± 1,15 h) were completely rigid before the end of the third hour. Muscle stiffness was maintained for approximately nineteen hours after CRA. The reversion process was initiated by the neck (19,68 ± 0,45 h) followed by the tail (20,30 ± 1,38 h), front limbs (22,23 ± 0,91 h), hind limbs (23,5 ± 1,16 h) and finally the TMJ at 24,91 ± 0,74 h. The process was completed before the end of the 26th hour after CRA. Corneal opacity begins 15 minutes after CRA (17,27 ± 6,17 min) and the eye becomes totally opaque during the first hour (48,64 ± 15,97 min). Mild sinking of the eyeball is observed before the end of the second hour after CRA (82,73 ± 35,19 min), evolving to total sinking of the before the end of the 22nd hour (1140 ± 129,67 min). Discussion and conclusionsThese results suggest that finding a mouse with rigor mortis, under typical animal house facility environmental conditions, indicates that the TOD occurred 4-24 hours ago. But, if animal house technicians perform a simple evaluation of TMJ, neck, FL, HL, tail movement and eyeball changes, is possible to understand if an animal died 2 hours ago (and sample collection is still possible) or if the rigor is already resolving (18-26 h post mortem).

PC23 Impact of bedding volume on group-housed laboratory female mice.

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Digging, burrowing and burying are essential components of mice`s behavioural repertoire and the animals are highly motivated to engage in these behaviours. Preference tests revealed that mice prefer a larger bedding volume in comparison to shallow bedding. Despite the fact that bedding is invariably used in the husbandry of laboratory mice, there is a lack of knowledge regarding the influence of bedding volume on animal welfare and experimental results.
Materials and Methods Female mice of the inbred strains BALB/cByJRj and C57BL/6NRj (36 mice per strain) are chosen for this study. After arrival, at three weeks of age, they are divided into groups of four. The animals are housed in Type III Makrolon cages in an open rack. Coarse-gained chips are used as bedding, the amount varies in accordance to the test group. The study is divided into two sets (one set per strain), testing three bedding volumes (0.5 l, 1.5 l, 6 l) at a time. Each set extends over 12 weeks. The animals are allotted into a total of 18 experimental groups (three groups per bedding volume and strain). Food and water intake as well as weight gain is assessed weekly. Two behavioural tests, open field and novel open recognition test are performed at eight weeks of age. Travel distance and freezing is recorded for each animal during the open field test, exploration time (of known and unknown object) is assessed for the novel object recognition test. In order to measure the animals` body temperature each mouse undergoes a short isoflurane anaesthesia and receives a subcutaneous transponder. The body temperature is obtained weekly during resting phase. At 14 weeks of age blood samples are collected for corticosterone measurement. At 15 weeks of age the mice are bled under CO2 narcosis. Blood samples are used to determine haematological and plasma parameters. Organ weights (heart, kidney, adrenal, spleen, liver) and final body weights are determined for all groups. Results Mice, which were kept on 6 l or 1.5 l of bedding had lower corticosterone values and higher body temperature in comparison to 0.5 l. The analysis of blood samples, organ weights and behavioural testing revealed strains differences for a majority of the parameters. Except for white blood cells, heart, kidney and adrenal weight there were no significant differences between the bedding volumes. Discussions and Conclusions The current results indicate that a larger bedding volume has a positive effect on the welfare of female BALB/cByJ and C57BL/6N mice without influencing a majority of clinical parameters. Therefore, it is more suitable to equip Type III cages with a bedding volume of at least 1.5 l rather than 0.5 l. According to this study, a large bedding volume can enhance animal welfare, especially during experiments that need to exclude any further environmental refinement.

PC24 Accelerometer in laboratory pig: tool for codification of pig behaviour during housing and quantification of activity during post-surgical recovery.

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Identification of abnormal behaviours and postures is essential to the recognition of signs of pain and distress in laboratory animals. Tools that contribute to the early detection of these behaviours will allow the application of immediate therapeutic measures. The use of accelerometers as a remote monitoring tool for movement quantification has been applied from free-ranging species to farm animals. Therefore, it also seems an obvious approach to identify behavioural changes in laboratory pig.
MATERIALS AND METHODS One female pig, enrolled in a porcine wound healing project, was included in this pilot study. During acclimatization, an acceleration logger (40x30x20 mm; 22 g) was attached to the back of the pig, between scapulae. The device included a temperature sensor and an accelerometer with orthogonally placed sensors for tri-axial acceleration, representing animal's dorso-ventral (z), anterior-posterior (y) and lateral (x) axis. Acceleration data, signal magnitude areas (SMA) and body temperature were recorded. Simultaneous video recordings were taken for categorization of active and resting periods and posture identification: i) standing; ii) sitting; iii) sniffing; iv) lying to the left; v) lying to the right. The corresponding x, y and z-acceleration data for each posture was exported and an algorithm was developed for posture detection. The algorithm was then applied for activity quantification during post-surgical period. Animals were allowed to recover from anaesthesia in their housing pen and SMA, acceleration data and body temperature were transmitted in real-time by Bluetooth every 60 seconds to a laptop localized in the monitoring room. RESULTS X-axis was chosen to predict lying to the right and left, and y-axis to predict sitting and sniffing. Standing was predicted by elimination and included activities such as eating, drinking and playing. After the surgical procedure, the animal took 20 minutes to acquire the stand-up position and body temperature continuously raised from 36ºC to 38,3ºC during the recovery period. During surgical recovery, the animal adopted all the positions pre-categorized, spending the recovery time as following: 11,6 % standing, 3,9% sniffing, 14,6% sitting, 32,92% lying to the left; 36,9% lying to the right. DISCUSSION AND CONCLUSION The use of accelerometry in laboratory pig allows the characterization of pig's activity during housing without human interference. Additionally, it provided real-time information on the time needed to acquire stand up position and the follow-up of body temperature variations during post-surgical recovery. The next steps of the project will be i) validation of the algorithm; ii) implementation of threshold alarms for the overall activity and iii) automatic detection of body postures by our software. These optimizations will allow the quantification of time spent in each posture in healthy pigs and consequently detect abnormal patterns in animals under pain or distress.

PC25 Air puffs as refinement of electric shocks for stimulation during treadmill exercise test.

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Endurance treadmill test is a convenient method for easily and systematically evaluate exercise performance and capacity in mice. Although mice are naturally prone to run, an aversive stimulation is required to force all animals to stay engaged in the test. This is typically achieved using mild electrical stimulation on a dedicated grid at the rear of the running belt.
Another system replacing the electric stimulation by air puff was newly developed. This system seems to be an ethical evolution in regards of the 3R's principles since the electrical aversive stimulus is replaced by a less noxious stimulus. Exhaustive endurance protocol was used to compare the two different treadmill systems. This test consists to expose mice to a long lasting middle intensity exercise protocol until they reach exhaustion. In addition of the exercise performance readouts, the adaptive mechanisms established to supply fuel to skeletal muscle during exercise and stress were respectively assessed by the analysis of biochemical parameters and markers of stress produced by the hypothalamic-pituitary-adrenal axis. The results obtained for the exhaustive protocol are comparable in terms of behavioral and physiological parameters (e.g. running distance, running time and physiological decrease of triglycerides and hepatic glycogen level). Interestingly, corticosterone and adrenocorticotropic hormone (ACTH) levels were similar between the two types of stimulation. These results demonstrate that air puff stimulation could be an interesting alternative for the replacement of the electric stimulation during an exhaustion protocol. However, the stress level does not seem to be reduced when using air puff stimulation.

PC26 Real-time monitoring of postoperative recovery in two inbred mouse strains by telemetry.

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In this study, we investigated the use of telemetry to identify differences in postoperative recovery in two different mouse strains. Telemetry allows monitoring physiological and behavioral parameters in real-time without the presence of the investigator in the vicinity of the animal. Body weight, heart rate, body temperature and activity of female C57BL/6J (B6) and C3H/HeJBir (C3) mice intraperitoneally implanted with a telemetric transmitter were examined.
Materials and methods:Telemetric transmitters, which are able to process heart rate (HR), electrocardiogram (ECG), core body temperature (BT) and locomotor activity (ACT), were implanted in B6 and C3 inbred strains. At the time of the surgical procedure, the mice were about 9 weeks of age and had a mean body weight of 21g +/- 2g. Animals were monitored and weighed daily for 14 days after surgery. The mice received analgesic-containing water that was flavored for better acceptance during 3 postoperative days. All data were recorded on conscious, unrestrained mice continuously for 24 hours. ECG, HR and core body temperature recordings from each animal were obtained at 30 min intervals, and the circadian patterns of Act, BT and HR were assessed. ResultsTransmitter implantation induced body weight reductions that were significantly greater in B6 mice compared to C3 mice. In B6 mice body weight required 15 days to recover to presurgical levels, whereas recovery of body weight in C3H mice occurred within 10 days. The mice had to recover from their surgical procedure for at least 4 days, before the animals started to demonstrate a normal diurnal rhythm of body temperature and activity. Core body temperature of the C3 mice was permanently lower than that of the B6 mice.BT and ACT were reduced in both strains directly after implantation. But at later regeneration time, B6 mice demonstrated 3 distinct peaks in BT and ACT during the dark phase while C3 mice showed continuous activity. Electrocardiogram evaluations showed quantitative differences in baseline HR between the two inbred strains. In C3 mice baseline HR was higher compared to mice of B6 strain. Discussion Our observations show differences in the recovery of body weight between both strains. The C3H mice attained presurgical body weight sooner than the B6 mice did. In our study, telemetric recordings demonstrated significant alterations in HR, BT and ACT after surgical procedures in the two different strains. With respect to HR, the C3 strain demonstrated a significantly greater daily average compared with B6 mice. The mechanism by which HR differs between strains may be related to the balance in sympathetic and parasympathetic autonomic control. HR regulation in B6 mice may be more vagally mediated compared to C3 mice. Conclusion In conclusion, telemetry was shown to be a useful tool for real- time monitoring of postoperative recovery in mice.

PC27 Severity Assessment by Utilization of the Time to Integrate to Nest Test (TINT) in a Mouse DSS-colitis Model.

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Severity assessment in laboratory animals is important for the implementation of the 3R concepts and is an integral aspect in current regulations of the EU. Severity assessment in colitis mouse models takes place by clinical observation. TINT is an easily applicable test detecting disturbed welfare by measuring the time mice need to integrate nesting material to an existing nest. Aim of this study was to investigate whether TINT can be utilized to assess severity in a mouse DSS-colitis model.
MATERIALS AND METHODS: Two different mouse strains (C57BL/6J (WT) vs. C57BL/6J.129S1-Cd14tm1Smg (Cd14-deficient; Cd14-/-) were treated with two different dextran-sulfate-sodium (DSS) doses (1% vs 1.5%) in drinking water for 7 days. Daily clinical assessment of animals was based on a previously published clinical score established to monitor severity in an experimental colitis model. Clinical parameters included loss of body weight, stool consistency and general clinical condition. TINT was performed using cotton-wool rolls and time which was needed for integration was measured. Initially a group size analysis for TINT was performed comparing cages with 1, 2, 3, 4 or 5 in-housed mice. RESULTS: A group size of 4 to 5 mice per cage resulted in consistent time intervals. Clinical observation of both mouse strains treated with 1% or 1.5% DSS revealed higher clinical scores and pronounced loss of body weight in 1,5% DSS treated mice compared to the 1% DSS treated mice. TINT time durations showed no dose dependent differences. When analyzing strain related differences we found increased clinical scores and body weight reductions as well as increased TINT time durations in CD14-/- mice compared to WT mice. DISCUSSION AND CONCLUSION: In conclusion, the results of the present study show that TINT is an easily applicable method for severity assessment in a mouse colitis model. The test was sensitive enough to detect CD14 related differences, although not dose dependent differences. As most consistent TINT results were gained in group-housed mice, we recommend utilization as an additional method substituting clinical monitoring of the individual mouse.

PC28 Transdermal fentanyl solution for non-invasive administration of post-operative analgesia in rats.

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Post-operative pain and stress should be treated with an appropriate analgesic strategy. However, parenteral drug administration calls for repeated injection, which is both stressful to the animals and resource demanding (1). Thus, development and validation of non-invasive regimens is necessary. The present study validated the analgesic effect of a transdermal fentanyl solution (Recuvyra®) in a rat model of post-operative pain. Materials and methods In total, 24 male Sprague-Dawley rats were used in the study. All animals, were subjected to a surgical incision in the right hind-paw as described by Brennan et al 1996 (2), except for one control group only subjected to surgery (n=6). Operated animals were treated once with transdermal fentanyl solution applied directly to the skin in a shaved area of the scruff, 1h prior to surgery, in doses of 0.1 (n=8), 0.33 (n=8) and 1.0 mg/kg (n=8). One control group was not treated with any analgesia (n=12). All rats were tested for nociceptive response in an electronic von Frey (EVF) test at 1, 6, 24, 48 and 72 hours post-operatively. In addition, animals were assessed daily with regards to body weight changes, welfare score, facial expression, and levels faecal corticosteroid metabolites (FCM). Results Treatment with transdermal fentanyl, in all doses tested, significantly reduced nociceptive response in the EVF test throughout the 72 hours of experimentation. The highest dose of fentanyl significantly reduced the body weight gain during the last two days of the experiment. The post-operative welfare scores showed a tendency towards improvement after fentanyl treatment compared to non-treated animals. Facial expressions were significantly improved in all fentanyl treated groups all test days post-operatively, while there were no differences in FCM levels. Discussion and conclusion Previous studies have shown voluntary ingestion of buprenorphine effective as a non-invasive post-operative analgesia in rats (3). Animals may though be reluctant to ingestion when in pain after invasive procedures. In addition, buprenorphine is not effective against severe pain conditions, why stronger opioids such as fentanyl may be indicated. The recent transdermal fentanyl solution Recuvyra®, which has been developed for use in dogs, was thus hypothesised to be applicable also in rats. It is clearly evident from the findings in the present study, that this transdermal fentanyl solution is an effective, non-invasive and long-lasting analgesic regimen in laboratory rats.

PC29 Evaluation of medetomidine-midazolam based anesthetic combinations in egyptian fruit bats (rousettus aegyptiacus).

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Bats, the only flying mammals, are increasingly used as animal models in the field of neuroscience, in addition to disease monitoring and ecological research. Handling of the bats often requires anesthesia for short or long duration. Several anesthetic protocols have been reported in bats species, usually based on case reports. The objectives of this study were to evaluate and compare the anesthesia and recovery induced with medetomidine-midazolam based combinations in Egyptian fruit bats.
Materials and methods Eight bats were randomly assigned by a crossover design to an anesthetic combination: medetomidine-midazolam-saline (MM-Sal), medetomidine-midazolam-ketamine (MM-Ket), medetomidine-midazolam-fentanyl (MM-Fen), medetomidine-midazolam-morphine (MM-Mor), or medetomidine-midazolam-butorphanol (MM-But) administered subcutaneously. Each bat was studied 5 times with at least ten-day washout period. Time to recumbency and recovery were recorded. The anesthetic depth, heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were monitored at baseline and every 10 minutes until bats were recovered spontaneously (started flying). If after 3 hours the bats did not recover, then reversal with atipamezole was performed. Results Mean induction times were 7-11.5 minutes with no significant difference between treatments. Following a short period of twitching (significantly less in the MM-But treatment), all combinations produced anesthesia, with significantly decreased HR (approximately 400 to 200), RR (approximately 140 to 36-65), and RT. The MM-Fen, MM-Mor, and MM-but resulted in significantly lower RR compared to MM-Sal, and MM-Mor had also significantly lower RR than MM-Ket. Mean recovery time was significantly shorter in the MM-Sal (88 minutes) in comparison to all other treatments, and it was significantly longer in the MM-But (159 minutes) in comparison to all other treatments. Atipamezole was administered only to 4/8 bats from the MM-But treatment. Discussion All evaluated combinations were effective, however MM-Ket produced longer anesthesia and caused less respiratory depression compared to opiate based combinations. Therefore this combination is recommended for general use. However, in painful procedures we consider adding a fourth analgetic agent. It is recommended to reverse MM-But combination.

PC30 What mouse behavior in an automated home-cage tells us

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Detailed analysis of mouse behavior is a central element of neuroscience research. It is well-recognized that behavioral read-outs are highly sensitive to arousal/stress-inducing interactions with human experimenters. Not surprisingly, over the last decades automated home-cage systems have become available for studying mouse behavior in the absence of human intervention. However, development of analysis methods to understand what the behavior of mice is telling us has been lagging behind.
Here, we review published and unpublished data of a number of recently developed protocols for PhenoTyper home-cages, designed to measure specific behavioral domains. In these protocols, typically single-housed male mice are observed with an overhead video camera, while stimuli and delivery of food rewards can be software controlled in real-time. Analysis of the first few days of spontaneous activity and sheltering behavior in these cages generates a rich dataset of behavioral parameters with tremendous statistical power, as used to differentiate common laboratory inbred strains [1, 2] (e.g. 129S1/SvImJ, A/J, C3H/HeJ, C57BL/6J, BALB/cJ, DBA/2J, NOD/LtJ, FVB/NJ, WSB/EiJ, PWK/PhJ and CAST/EiJ), as well as dozens of mutant mouse lines with targeted deletions of brain-expressed genes (e.g. [3, 4]). Longitudinal and multiparametric assessment of activity patterns contributed significantly to detect the effect of compounds (e.g. [5][6]). Similar multiparametric assessment of activity patterns indicated specific changes in mouse models of neuromuscular diseases such as Amyotrophic Lateral Sclerosis (ALS), as well as mice experiencing post-surgery pain/distress. Protocols for measuring anxiety-related behavior have used subtle anxiogenic stimuli such as an open/elevated feeding area [7] and a bright light directed at the feeding area [8], and were pharmacologically validated using anxiolytics. Currently available

cognitive tasks in this home-cage system include avoidance learning, using a subtle negative reinforcer (illumination of a shelter area [9]), and more complex instrumental learning tasks in which mice learned to obtain food rewards [10; unpublished data]. The latter appeared highly sensitive in detecting learning deficits induced by pharmacological challenges, lesions of specific brain regions and in mutant mouse lines with known synaptic dysfunction, such as the APP/PS1 transgenic mice that recapitulate part of Alzheimer's Disease symptomatology [unpublished data]. Taken together, more than one decade after the introduction of automated home-cages equipped with integrated video tracking, observation of mouse behavior can yield detailed, reproducible data on aspects of cognition, anxiety, motor function and pain/distress relevant to neurological and psychiatric diseases.

PC31 Buprenorphine for pain relief in mice: Administration via drinking water and repeated injections

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Buprenorphine is the opioid analgesic most commonly used in mice. However, to maintain therapeutically effective serum levels, repeated injections are required. To overcome negative aspects of restraint and injection, oral self-administration is a promising alternative but has been criticized to be unreliable. Here we analyze voluntary intake of buprenorphine via drinking water as well as drinking water/injection combinations for their reliability to achieve effective drug supply in mice.
Female C57BL/6J mice were assigned to one of five groups: a) naïve/no treatment (N); b) buprenorphine administration via drinking water (W); c) buprenorphine administration via two subcutaneous injections during light, and via drinking water during dark phase (IW2); d) buprenorphine administration via three subcutaneous injections and drinking water for 24 h (IW3) or e) surgery plus buprenorphine administration via three subcutaneous injections and drinking water for 24 h (S). Drinking frequency, activity, water and food intake, body weight, blood serum concentrations of buprenorphine and behavioral pain indicators were determined. Administration of buprenorphine resulted in an increase of home cage activity and a decrease in body weight (n.s.). Food intake decreased significantly in IW2 and IW3 and after surgery, compared to naïve mice (IW2: $p=0.001$; IW3: $p=0.0253$; S: $p\leq0.0001$). Water intake was not decreased due to buprenorphine treatment or surgery. All treatment groups showed mean serum concentrations higher than the targeted value (> 1 ng/ml) throughout dark phase. Sporadic drinking events and consequently highly variable individual serum concentrations during light phase suggest the use of a combination protocol (IW3: 24 h water administration + injections every 4h during light phase), that proved to result in continuous therapeutic mean and individual serum concentrations and minimization of pain indicators after surgery (S).

PC32 The consequences of premedication with buprenorphine, butorphanol or tramadol on anaesthesia safety induced with IV alphaxalone

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Anaesthetics and analgesics have to be co-administered in order to allow for potentially painful interventions to be carried out. However, such co-administration may exercerbate side effects. There are no published data on cardiorespiratory effects of analgesics in combination with anaesthetics in marmosets (Callithrix jacchus). We investigated clinical and physiological effects of intravenous alphaxalone in combination with analgesic premedication with buprenorphine, butorphanol or tramadol.
Material and methods A prospective, blinded, crossover study in nine marmosets was designed (four male, five female, mean body weight 391 g, mean age 3.70 years) and Ethics approval by the Animal Experiments Committee (DEC) of the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands) was obtained prior to the commencement of the study. All marmosets originated from and were housed at the BPRC. The marmosets were socially housed indoors with a same-sex buddy.One hour prior to anaesthetic induction with intravenous alphaxalone (16 mg kg-1), the marmosets were premedicated with subcutaneous meloxicam, intramuscular atropine and one of the following intramuscular analgesics: buprenorphine (20 µg kg-1), butorphanol (0.20 mg kg -1) or tramadol (1.5 mg kg -1). During anaesthesia, the following physiological parameters were recorded every 5 minutes: respiratory frequency, pulse rate, rectal temperature, haemoglobin oxygen saturation, and arterial blood pressure. Judged were palpebral reflex, withdrawal reflex and muscle tone. Qualitative scores for induction and recovery were allocated and adverse events recorded. Duration of induction, immobilisation and recovery were recorded. Results We observed of an unexpectedly high incidence of apnoea in the buprenorphine group (8 out of 9 marmosets). One apnoea even lasted for 63 minutes. Significantly less adverse effects were observed in the butorphanol group (2 out of 9 animals). No such adverse effects were observed in the tramadol group. Similarly, intravenous alphaxalone without intramuscular analgesics showed no cardiorespiratory abnormalities. There were no significant differences when comparing the baseline physiologic parameters (excluding the apnoeic animals) between the protocols. Discussion and conclusions Optimisation (refinement) of anaesthetic protocols and appropriate analgesia means implementation of the 3R's. In view of the severe respiratory complications encountered, we advise caution when considering pre-anaesthetic use of buprenorphine at the dose used in this study in combination with intravenous alphaxalone in marmosets. The combination of tramadol followed by intravenous alphaxalone instead can be considered safe, reliable and applicable for general anaesthesia in marmosets. Testing for analgesic efficiency was not part of this study.

PC33 Carprofen caused severe adverse effects in rats – A case report.

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During the last decade several organisations recommended adequate pain management for animal welfare. In rats Carprofen is commonly used for perioperative analgesic treatment. Thereby a multimodal approach is frequently used in order to enhance the analgesic effect. Therefore, we combined three different analgesic drugs (butorphanol, carprofen, metamizol [dypirone]) to improve perioperative analgesia for an orthopaedic study in rats.
Twenty-seven female Lewis rats aged 7 to 10 weeks underwent surgery for implantation of a pin consisting of approved implant material into the right femur. During the course of this underlying study no complications could be observed. Isoflurane in O2 was administered for general anesthesia. For analgesia, all animals received a single subcutaneous injection of Butorphanol (1mg/kg) before surgery. To guarantee a specific musculoskeletal analgesia a subcutaneous injection of Carprofen (5mg/kg) was given directly after surgery and half of the dose daily for the first three postoperative days (group 1). Due to the observation of severe gastrointestinal side effects, the analgetic treatment was changed to injection of Carprofen 2.5mg/kg in one group (group 2) and Metamizole (Dipyrone) 0.5g/l drinking water for another group (group 3) in addition to Butorphanol. RESULTS Within the first three days animals of group 1 showed a strongly reduced general condition indicated by ruffled fur, prostration and dehydration. Two rats died within 24h hours after showing the first clinical signs and subsequent necropsy revealed peritonitis and inflammatory adhesions. By histological examination severe ulcerations of the small intestine, ranging from erosions of the mucous layer to perforated intestinal walls could be observed. Further three animals of the same group had to be euthanized within 5-7 days after the first administration of Carprofen and Metamizole. Three animals from the five animals in group 2 developed the same pathological changes as seen in group 1. For all nine animals of group 3 the general conditions as well as histology were unaffected. CONCLUSIONS Carprofen alone and in combination with Metamizole is an established analgetic regime used for laboratory animals. However, findings in this study suggest the use of Carprofen in rats with caution. To eludicate the gastrointestinal effect of Carprofen in rats controlled studies will be needed.

PC34 How to determine severity degrees for genetically altered rodents?

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Genetically altered animals are frequently used research models with continuously increasing numbers. Apart from its scientific value, the genetic alteration can compromise animal well-being. However, the large variety of phenotypes is challenging when it comes to severity degree determination. A guide on severity classification will therefore largely contribute to the harmonisation of severity assessments of genetically altered mice and rat lines throughout Europe.
Materials and methods: The breeding of genetically altered animals needs to be authorized by the competent authority within the scope of the European Directive 2010/63, if a harmful phenotype is likely to occur. Hence, the severity classification plays an essential role in pro- and retrospective severity assessment. Since 2013, severity assessment and classification of genetically altered laboratory animals has to be established at all European research institutes. The German Federal Institute for Risk Assessment developed guidelines for a basic welfare assessment [1]. According to these guidelines a final assessment form has to be filled in for each established line containing the severity degree, a description of the phenotype and refinement recommendations. The Arbeitskreis Berliner Tierschutzbeauftragte (Working Group of Berlin Animal Welfare Officers) collected and reviewed severity classifications of genetically altered mouse and rat lines. We compiled data from Berlin's principle research institutes whose in vivo research uses almost exclusively mice and rats with genetic alterations and who keep some 180.000 rodents in total. Data of the years 2013 through 2015 has been included. Results: The Guide on severity assessment and classification of genetically altered mice and rat lines contains examples of symptoms and syndromes caused by genetic alterations. Examples are assigned to a particular severity category (none, mild, moderate, severe) including recommendations for refinement strategies. These classifications are based on a consensus reached by experts in veterinary medicine, laboratory animal science, and animal welfare. In addition, recommendations of the British Home office [2], and the European Commission Expert Working Group on severity classification [3], as well as selected scientific publications on phenotypes have been taken into account. Discussion: This collection of severity classifications will serve as guidance in the complex process of severity degree determination and assignment. Animal welfare bodies and researchers are encouraged to contribute information on phenotypes which will be incorporated into the collection. The list will be reviewed periodically in order to provide a large collection of different syndromes and clinical symptoms and will be a way to increase the harmonisation of the approach across scientific research institutes.

PD1 Mouse Gut Flora Increment in SOPF Isolators

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Charles River maintains SOPF animals mainly in isolators. The mice were implemented originally with defined flora specific to CRL (CRAS Flora1 and SOPF flora2). Over the years and throughout many animals' transfer between isolators, an increment of those defined flora is observed between the opening and the closing of those isolators. The purpose of this study was to compare the gut flora between isolators from different CRL sites and compare the aerobic bacteria "drift" in the gut flora.
Material and methods: Aerobic gut flora is evaluated by bacteriology every 6 weeks in SOPF isolators. The submitted sample types were feces (8 pellets), water, and dust from the floor. The samples were plated on 3 media (CAN, TSS, PYO from BioMérieux) and incubated for 24 to 48 hours at 35 °C. Bacteria isolates were identified by biochemistry methodology (VITEK®). We retrospectively compiled results of bacteriological screening over 141 isolators from 3 Charles River sites representing over 4,000 bacteriology tests and a period of 4 years (from 2010 to 2014). Results: The 3 Charles River sites showed different flora. One site has a larger flora with 7 aerobic bacteria, whereas the others have 4 aerobic bacteria. The initial CRAS + SOPF flora was observed in site A, whereas the others sites showed only 1 or 2 bacteria from those initial flora. The flora "drift" detected over time in isolators (all sites compiled) represented 30 different bacteria genus, with a maximum of 7 different bacteria genus in one isolator. Some of these bacteria (6 genus) are common to all sites, such as Acitenobacter sp and Stenotrophomonas sp, and others (3-6 genus) are very specific to each site, such as Rhodococcus sp, found only in site C, and Serratia sp, found only in site A. Conclusion: Despite maintaining mice in a highly controlled environment such as isolators, a minor aerobic gut flora increment can observed over time. By following this gut flora "drift," Charles River can maintain mice with a minimal flora to guarantee the SOPF health status in isolator.

PD2 Introduction of the strain GDV Yale in the production of a bio-reagent for the development of a Theiler virus immunodiagnostic kit of SPF mice

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Mouse Encephalomyelitis virus or Theiler´s disease(TEMV) is one of the most studied entities in the laboratory mouse, because of the prevalence that develops once introduced into breeding colonies. As aspects relevant for the diagnostic kit, it is the determination of the antigen presence and purity to be used in the preparation of the diagnostic kit. The objective was to describe the procedure followed with the strain GDVII Yale in the development of a Theiler virus immunodiagnostic kit of mice.
Materials and methods: The strain came from the Central Institute for Laboratory Animal husbandry, Hannover, Germany. Its use was aimed at obtaining biological reagents such as antigen-positive sera and negative patterns. For multiplication of the strain, it was used as substrate, the BHK-21 clone 15 (ATCC) line. Results: The cytopathic effect was observed for 72 hours and the optimal time of viral cultivation affected more than 75% of the monolayer. Purification of antigen is performed in a single density gradient of cesium chloride. Discussion and Conclusions: As criterion of purity, it was taken into account the biological activity and testing of SDS-PAGE of Laenmly, showing the structural proteins VP1, VP2 and VP3, with value for the diagnostic kit. It is concluded that the procedure followed with the strain GDVII Yale, allowed the development of a Theiler virus immunodiagnostic kit of mice in the CENPALAB.

PD3 Determining the presence of antibodies to murine virus in breeding colonies of gnotobiotic mice

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Among the species of laboratory animals produced in CENPALAB, rodents (rats and mice) bred and maintained under controlled conditions are most commonly used in biomedical research. Virological control is essential to achieve a health hygienic quality required for these biomodels. The study aimed to detect the presence of specific antibodies to murine virus in a breeding colony of gnotobiotic mice, CENPALAB.
Materials and methods: 18 viruses were evaluated: Sendai (Sd), Tiny Virus (MVM), Murine Hepatitis Virus (MHV), Pneumonia (PVM), Cytomegalovirus (MCMV), Adenovirus murine (MAD), EDIM, Ectromelia (Ectro), Lymphocytic Choriomeningitis (LCM), Parvovirus (Parvo), Norovirus, NS-1, Murine Encephalomyelitis (TEMV), REO-3, POLYOMA (Poly), Hantavirus, Thymus and Pneumonitis. Serological systems of two types, indirect ELISA and IFI, were used from the commercial firm Charles River. The study included a total of 2000 samples, those from BALB/c, BALB/c XID, BALB/c Nu, ApoE and C57BL/6 lines. All mice were in the age of development. Results: The results of viral prevalence were: Norovirus 52,74%, MHV 47,19%, MVM 26,85%, NS-1 25%, Parvovirus 4,61%, Sendai 1,57%, LCM 0,98%, MAD 0,58%, MCMV 0,49% and EDIM 0,39 %. These values indicated that the most prevalent virus in mice was Norovirus followed by MHV ($p < 0.05$), being their infection higher than the MVM, NS-1, Parvovirus, Sd, LCM, MAD, MCMV and EDIM ($p < 0.05$). Pneumonia virus appeared similar to Parvovirus, but higher than the LCM ($p < 0.05$). Discussion and Conclusions: The prevalence of virus samples tested in mice CENPALAB breeding colonies showed the same characteristics present in reports of these viruses in animal facilities of Venezuela, Brazil, Europe and the United States. It was evaluated the presence of specific antibodies to murine virus in a breeding colony of gnotobiotic mice, CENPALAB.

PD4 Microbiological monitoring effects of the cross-fostering in conventionally bred rats

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Rat pups acquire passive immunity from their mothers through placenta and colostrum which protect them against various infectious agents in the first days of their lives.Fostering is routinely used in breeding colonies,and it is important for supporting pups whose dams die during lactation or while giving birth. We aimed to compare the serological profiles of pups reared by their biological mothers and the ones,which were transferred to foster dams after birth,with regard to certain viral agents.
Materials and methods: Not related virgin Wistar albino female rats (n=6) were bred. Then the dams divided into two groups. The day of delivery for the pregnant rat is recognized as the postnatal day 0 (P0). The pups born from Group 1 [P(0), P(21),P(60)] pregnant rats were housed with their biological mothers. In Group 2 [P(1), P(21),P(60)], however, P(1) consisted of pups housed with their biological mother for 24 hours while P(21)

and P(60) consisted of pups transferred to the their biological mother immediately at birth. At the end of the experiment, blood was collected by cardiac puncture. The presence of antibody was tested with ELISA for Kilham's Rat Virus, Lymphocytic Choriomeningitis Virus, Murine Adeno Virus Type 1, Type 2, Rat Parvo Virus, Reovirus Type, Sendai Virus, Sialodacryoadenitis Virus / Rat Coranavirus, Toolan's Virus, Theiler's Murine Encephalomyelitis Virus. Results: Antibody response against Lymphocytic Choriomeningitis Virus, Rat Parvo Virus, Sendai Virus and Toolan's H1 viruses was not detected in dams and pups of all the groups, whereas high rate (45%) of antibody positivity was observed in response to Kilham' Rat Virus(KRV). No statistically significant difference was determined between the pups left with their biological mother and with the foster mothers, except for Murine Adeno Virus type 1, type 2(MAD 1&2), Reovirus Type 3(REO-3) and Theiler's Murine Encephalomyelitis Virus(TMEV). Discussion and conclusion: It was conspicuous in terms of REO-3 that for the pups it was essential to stay with their biological mothers and nursed during the first 24 hours. As a result, foster mother usage in breeding laboratory animals is found to be beneficial against a variety of viral agents for the pups particularly in the late periods of their lives and we suggest to continue this implementation in routine laboratory practice whenever necessary. Keywords: Cross-fostering, Rat, Microbiological monitoring, Viral agents

PD5 Comparison of hematological parameters in blood samples from the retro-orbital plexus and vena cava in Wistar rats

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Clinical pathology ranges are important factors to various investigations, especially for toxicity studies. Numerous studies have been performed examining the effects of blood collection site on hematological parameters in rats. However, they were performed either a long time ago or under ether anesthesia. The aim was to assess the equivalence in blood samples from the Caudal Vena Cava(VC) and those from Retro-Orbital(RO) in order to ascertain if sampling from VC could replace RO before necropsy
Animals Forty healthy male Wistar rats 16 weeks old were assigned to two groups. Group A RO, and Group B VC, N=20 each. All samples were collected in the noon. Blood sampling methods For blood collection in both groups, animals were anesthetized with Sevoflurane. The VC is accessed surgically and the blood was cannulated directly by placing a 21G winged needle. For the RO procedure, a heparinized Pasteur pipette was used to collect blood from the orbital sinus plexus. All blood samples were collected in 0.5ml tubes with Dipotassium EDTA and analyzed within 1 hourHematological parametersThe analysis of the samples was conducted with the ProCyte Dx Hematology Analyzer–IDEXX. The hematological analysis included the following parameters: red blood cells(RBC), hemoglobin(HGB), hematocrit(HCT), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC), red blood cell distribution width(RDW-CV), reticulocytes(RET), immature reticulocyte fraction(IRF), low fluorescence reticulocytes(LFR), middle fluorescence reticulocytes(MFR), high fluorescence reticulocytes (HFR), platelet count(PLT), mean platelet volume(MPV), platelet hematocrit(PCT), white blood cell count(WBC), Neutrophils(NEUT), Lymphocytes(LYMPH), Monocytes(MONO), Eosinophils(EO) and Basophils(BASO)Statistical analysisThe two one side test(TOST) was used, which is a type of test primarily used to validate bioequivalence. A(1–2α)x100% confidence interval(CI) was selected, with significant level a=0.05. Thus, a 90% CI was used. The methods were considered equivalent if the 90%CI is contained within the interval (-δ,δ). For this analysis, the equivalence margin was set at δ=1 pooled SD from each hematological parameterResultsThirteen from 21 parameters revealed equivalence such us RBC, HGB, HCT, MCV, MCH, MCHC, PLT, PCT, WBC, NEUT, LYMPH, MONO, EO from which PLT(0.08%) displayed the smallest and MONO(16.39%) the largest percentage difference. No equivalence was found for RDW-CV, RET, IRF, LRF, MRF, HFR, MPV and BASO from which RDW-CV (3.52%) displayed the smallest and HFR (42.34%) the largest percentage differenceConclusionComparison between VC and RO revealed equivalence in the majority of the complete blood count parameters evaluated, but RO data tend to yield slightly lower mean values, although VC data tend to have a better coefficient of variation. These results indicate that blood sampling from VC can be used as an equal alternative to RO blood sampling.

PD6 Endogenous infection caused by Streptococcus agalactiae in KK-Ay mice

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KK-Ay mice, also known as Yellow KK obese mice, are widely used as models for type 2 diabetes. Streptococcus agalactiae (Group B Streptococcus) infection in healthy human adults is extremely uncommon and is almost always associated with underlying abnormalities, such as diabetes mellitus or compromised immune defense.
Spontaneous group B Streptococcus infections occurred in KK-Ay mice, an animal model of type 2 diabetes. Twenty-two clinically ill female mice from 5 to 27 week-old were observed: 18 cases of various parts of body swelling such as facial and lumbar swelling, 2 cases of torticollis, 1 case of head enlargement and 1 case of moribund symptom. At necropsy, the swelling was the localized abscess, the head enlargement mouse had the retained exudate in the uterus and the moribund mouse showed the atrophy of kidneys. S. agalactiae was all isolated in pure culture except for 2 cases: the abscess from the face was coinfected with Klebsiella pneumoniae and the retained exudate in the uterus was coinfected with Staphylococcus aureus. From the oral cavity and feces in normal KK-Ay mice, S. agalactiae was isolated.S. agalactiae causes a potentially clinically significant spontaneous infection in a diabetic mice model.

PD7 IMPACT OF THE USE OF GNOTOBIOTIC ANIMALS FOR BIOMEDICAL RESEARCH IN CUBA

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This paper's main purpose is to demonstrate the development of the science of laboratory animals in Cuba in the last 29 years, its new challenges and perspectives and behavior show the use of animals for biomedical research.
Results: Cuba began to take the first steps with the approval of the project in 1982 and in 1986 CENPALAB obtaining the first gnotobiotic animals. From the year 1994 on the CENPALAB demand satisfaction to all the centers that make up the National System of Laboratory Animals (SNAL) with SPF hygienic and sanitary quality is achieved, mice being the most popular animal model, which reached occupy 95.7% of the total number of animals delivered to users centers. Rodents have delivered 5 species and more than 14 lines which have SPF hygienic and sanitary quality, eliminating rodents conventional sanitary quality, which contributes to the development of biotechnology and pharmaceutical and medical industry these animal models that meet international standards and guarantee the reliability and reproducibility of the experiments involved. Conclusions: Cuba today has a system of laboratory animals at the level of the industrialized countries; there is human resources which are in constant training to meet the new challenges of the science of laboratory animals

PD8 The use of controlled hydrated diets in the context of preclinical and regulated trials made on dogs

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Pre-clinical studies muts be carried out, in particular, trials with products that are still being developed. Certain products may provoke problems in the way of feeding. An alternative to the standard diet pellets' which still maintains the quality of a controlled diet is, then mandatory. The availability of new scientific data on animal testing is governed by the principles of Replacement, Reduction and Refinement, linked to the major factors influencing the animal's welfare (the [3]R's).
Nutrition is one of the most important factors when it comes to the successful conduct of a pre-clinical GLP or non-GLP trial. The reduction in diet intake is often linked to the administration of a tested drug that provokes pain and/or stress in the animal, even when the standard, as the 326C diet, is rich in lipids and very appetising. It is also one of the first early warning signals, quickly followed by the appearance of severe clinical signs (a rapid loss in body weight, dehydration, etc.). This reflection stems from a study carried out on dogs with a low diet intake from the moment of first administering the trial product, such that recourse is made to a moist standard common diet, one that is not subject to controls or compliant with GLP and sourced from general distribution networks.In the context of some types of trials on dogs, in which the administration of certain compounds leads to clear loss in appetite and entails a possible premature sacrifice made of the animals concerned, on reflection, it seemed imperative for us to remedy this lack of appetite by recourse to a hydrated and controlled diet from SAFE®, in order to avoid that premature loss of animals. The GELDIET 125 is a complete nutritional solution, which allows you to combine the hydration and feeding of animals in one product, easily done in terms of nutritional uptake. The GELDIET 125 is made up of 73 % water and 25 % 125 dog diet. The aim of these trials was to provide an alternative to our standard diets, whilst preserving all the qualities of a controlled diet, equally to reduce the side-effects on the animal's welfare, thus meeting the requirements of the 3Rs and the GLP.

PD9 Husbandry and care of minipigs for pre-clinical study in Russia

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In Europe, minipig are widely used as an animal model in toxicology studies. The minipig presents a favourable profile as a non-rodent toxicology model, in terms of the similarity to man and also in terms of applicability to different study types. Our organization adhere to the principles of 3Rs and Directive 2010/63/EU. To improve the work in accordance with the principles of 3Rs 3 years ago we started breeding minipig as an alternative to rodents.
The minipigs animal facility should have the necessary space to accommodate the animals. For the calculation of the minimum floor area, we used the local and European standards. The minipigs have highly developed social behavior and usually we keep the groups of females, since animals can communicate and play with each other. Males are contained individually. Great importance in the minipigs animal facility should be given to the materials making up the room, especially the floors. For pigs on farms in most cases use slatted floors made of concrete, iron coated with rubber, plastic. Due to the large diameter of the cells and the high risk of trauma most of the specialists don`t recommend using these floors for minipigs. Searching quality and suitable materials for floors it`s very difficult challenge. Coatings for content minipigs must possess many properties: aren`t toxic for animals and humans, non-slippery, durable, easy for cleaning. In our minipigs facility the floors are covered in a special coating made from natural ingredients rubber and sand. The combination of these components has many advantages for the health of animals, the absence of sliding hooves, hoof stitching stratum corneum, availability of materials, safety for animals. In our minipigs facility we additionally use erithema lamps, the spectrum of action which has a positive effect on the growing and development of pigs and piglets, and improves the digestibility of calcium and vitamins. As a bedding material and enrichment of the environment, we use wood chips and hay. For reducing the concentration of ammonia in the air content and microbial contamination of the boxes we use natural powder driers, which is mixed with bedding material. As the enrichment of the environment and for the welfare of the animals we use plastic balls of different sizes, wooden poles, depending on the time of year using grassy lawns. It stimulates locomotor activity, improves motor skills of minipigs. For the health of the animals is necessary to combine a variety of factors, the most important proper maintenance and welfare of animals. The health status of animals takes an important role for research. Using minipig for pre-clinical studies will improve the safety of drugs and will protect volunteers in phase 1 clinical trials.

PD10 Challenges in Pasteurella pneumotropica diagnostics

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Pasteurellaceae comprise a large and diverse family of gram negative bacteria that are frequently cultured from laboratory rodents and rabbits. Most of them are opportunistic pathogens that can cause a wide range of clinical problems in immune competent and immune deficient animals. It is known that a correct classification of Pasteurella pneumotropica is difficult which makes it challenging to maintain the 2014 FELASA guidelines.
In 2014 the recommendation of the Federation of Laboratory Animal Science Associations (FELASA) to test rodents and rabbits for Pasteurellaceae has been changed to test for Pasteurella pneumotropica only. In the 2014 recommendations it is already mentioned that it is difficult to correctly classify Pasteurella pneumotropica, resulting in misclassification and discrepancies of test results between test laboratories. The problem relies on the presence of V factor dependent and independent Pasteurella pneumotropica strains which cannot be classified using different biochemical assays like API 20NE and NH system. In addition, most of the PCR's which are currently applied in diagnostics restrict to Pasteurella pneumotropica biotype Heyl and Jawetz strains. It appears that V factor dependent strains are often missed at screening, although these Pasteurella pneumotropica strains are frequently found in the respiratory tract of laboratory mice and rats at routine health screening in our laboratory. We developed a Pasteurellaceae PCR, and in addition, species specific PCR's for further identification of Pasteurella pneumotropica including V factor dependent strains and Actinobacillus muris. Testing a large number of diagnostic samples made it clear that misclassification of Pasteurella pneumotropica strains is a problem. Changing FELASA 2014 recommendation might have as unwanted effect of unknowing introduction of Pasteurellaceae in animal facilities.

PD11 Mitigation of Risks from Rederivation using In Vitro Fertilization and Embryo Transfer to Eliminate Pathogens from Research Mice

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Mouse strains are frequently rederived because they are being newly introduced into an animal facility or because of issues of contamination. Thus, the process of rederivation has become an important part of the strategic management of research animal facilities. This poster will demonstrate the risks we have identified in our rederivation process at The Jackson Laboratory and the methods of risk mitigation we have undertaken to reduce the likelihood of contaminating mice during this process.
Introduction of new mouse strains into an animal facility or rederivation of existing mouse colonies are risky undertakings requiring strict control measures. In vitro fertilization and embryo transfer (IVF/ET) after thorough washing of the embryos are the methods most frequently employed to rederive research mice and assure maximum health of the resultant offspring. The standard practice for rederivation of research mice is to produce embryos, wash embryos, implant those embryos into foster mothers, raise the fostered pups and release the litter at weaning In terms of risk assessment, this process starts with the risk of using contaminated gametes and ends with the risk of releasing contaminated pups. Our analysis of this process identified two significant areas of concern: 1) Contaminated animals could be present without being detected until after contamination has spread. The time from embryo implantation to weaning, when the mother can be tested for murine pathogens is about 6-7 weeks. This means there is a 6-7 week delay in knowing whether the embryos transferred were contaminated and whether these contaminants spread to the foster mother and possibly to other mice within the same mouse room. 2). The focus of rederivation is on the elimination of pathogens present in the original colony that could be passed to the offspring. However, there is also risk of contamination during the rederivation process from exposure and contamination in the room environment. As a result of this analysis we have implemented new processes to mitigate the risks identified. A qPCR based testing approach has been developed and validated for testing the final embryo wash fluids for mouse pathogens to mitigate the risk that pathogens are transmitted from embryos. Furthermore, on a weekly basis during the 6 week period from embryo implantation to weaning of litters, fecal samples are randomly selected from foster mother cages to monitor for pathogenic bacteria that could potentially be acquired from the room environment. Additionally, at weaning, groups of foster mothers with weaned litters are necropsied and thoroughly tested via serology, parasitology, PCR and bacterial culture for the presence of infectious agents that could have potentially contaminated mice from environmental sources. Taken together, these steps are a novel approach to addressing the risks at each stage of the rederivation process and have proven very effective in mitigating these risks.

PD12 Life Cycle Assessment of an Animal Facility: A comparative study between washable & disposable cages

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In the name of sustainability, an Organization Life Cycle Assessment (LCA, a multi-stage approach from the cradle to the grave) of the EPFL animal facility was performed in collaboration with Quantis (www.quantis-intl. Ch). After having evaluate the environmental impact of our facilities (data not shown), we present here a simulation of the replacement of all washable cages currently used by disposal cages to assess which alternative would be the best in terms of environmental impactThe monitoring of the EPFL animal facility during [2012] included the following activities: administration and back office (building and energy, commuting and business travels), mice husbandry (cage & rack production and distribution, litter and feeding, building and energy consumption), cage, rack and other material washing & disinfection, ventilation, import and export of animals (transportation), scientific procedures (material, building and energy) and waste management. Our animal house hosted a single species, mice ([9'000] individually ventilated cages). There were a specified pathogen free, a conventional and a phenotyping unit. P[1] & P[2] labs were set up inside the animal house. All cages and racks were washing and autoclaved before entering the housing units. To compare the the washable cage scenario, we simulated two scenarii for the disposable cages; scenario V[1]: same disposable cage change frequency of [10] days as for washable cages; scenario V[2]: longer disposable cage change frequency of [2] weeks (cage bottom) and [1] month (lid &feeder). We considered three environmental impact factors: climate change, human health and ecosystem quality. Results and conclusions will be presented; they are limited to the objectives, goal, scope and assumptions defined in this study, and are valid only for the specific case of the EPFL animal facility. However, our study indicates that LCA is an interesting tool to assess "greeness" or environmental impact of any animal facility design, new or renovated.

PD13 Syphacia obvelata and Radfordia affinis infection in mice; treatment strategy, implementation of a new health monitoring system and establishment of improved quarantine procedures

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In 2014 we experienced an infection with pinworms. Subsequent health monitoring revealed positive findings of Radfordia and Tritrichomonas. Activities were initiated in an attempt to eliminate the infections and to prevent future similar events: -Fenbandazole treatment in drinking water (0.3 mg/ml, 5 weeks)-Selamectin treatment topically (1.2 mg/animal, 3 weeks)-Establishment of a new quarantine facility-Revision of staff access-Change from conventional serology to PCR based monitoring
Materials & methods: Drinking water containing 0.3 mg/ml fenbendazole (Panacur® Vet 10% Oral Suspension) was made by mixing 0.75 ml of Panacur® Vet with drinking water in 250 ml bottles. Precipitation in drinking water was minimized by handling the bottles on regular basis. Mice were treated for one week followed by one week without fenbendazole. This was repeated 5 times consecutively. Topical treatment of mice with selamectin (Stronghold® Vet, selamectin 120 mg/ml) was performed by dripping 0.01 ml of Stronghold stock solution in the neck using a micropipette (1). Every second week all mice more than 8-10 days old were treated once. Treatment was repeated 3 times on consecutively weeks. Syphacia obvelata infection was initially diagnosed by perianal tape tests from clinically infected mice and subsequently also by PCR on feces samples. Radfordia affinis infection was initially diagnosed by microscopy of fur/skin smears from clinically affected mice demonstrating live fur mites. Subsequently, fur mite infection was diagnosed by PCR analysis on fur swabs and swabs from exhaust manifolds of IVC (2). Results: Following fenbendazole treatment of mice all subsequent screenings of animals by perianal tape tests and PCR analysis on feces samples have been tested negative for pinworms. Treatment of mice with selamectin has led to complete eradication of fur mites in the facility, and all subsequent

screenings by microscopy and PCR on fur swaps and from exhaust manifolds of IVC have been negative. Treatment strategies for eliminating Tritrichomonas muris have not yet been established, and the protozoa is diagnosed on irregular basis by PCR and microscopy on intestinal smears. Discussion and conclusion: Previously new animals were accepted after evaluation of a recent health monitoring report only. Due to an increase in mouse population, with a growth from an average population density of 2.594 mice in 2008 to 4957 mice in 2012, the number of imports and staff movements has increased drastically, resulting in a higher risk for acquiring infections. The infections with pinworms and fur mites were successfully treated, and successive health monitoring has demonstrated continuous absence of these pathogens. Tritrichomonas muris is to a wide extent regarded as a commensal in the intestinal lumen with minimal side effects on mice (3), and so far, the infection has not been related to clinical or pathological changes in mice in the facility.

PD14 Animal welfare and health can be achieved through treatment and safe housing and management procedures: a case of eradication of Klebsiella spp from mice colonies in a conventional animal facility

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To guarantee animal welfare and research quality, animal facilities need a health monitoring program able to respond to internal needs as indicated by FELASA recommendations. Our study presents the results of the containment, in a conventional facility, of commensal pathogens as Klebsiella, a potential issue for immunocompromised animals, through pharmacological treatment with Enrofloxacin and appropriate working and sanitization procedures.
Our animal facility could be considered at high risk of contamination because it is a conventional facility without an autoclave that has to deal with a frequent inlet of animals, often transgenic, from several facilities throughout the world. Additionally, research personnel often enter the animal facility in addition to animal care staff. All these potentially risky features led to the extension of our health panel to include infrequent pathogens as Klebsiella spp. From February 2012 to December 2015 we received, from different animal facilities, 254 mice of different strains positive to Klebsiella spp. All the animals were allocated in quarantine using ventilated cabinets in conventional cages with filter top and environmental enrichment. Of those 254 mice, 141 have been pharmacologically treated with Enrofloxacin (0,2mg/ml of drinking water for 2 weeks), while 113 have not been treated for experimental reasons. Check-up tests have been performed on biological samples from each cage at the end of the 7-days washout period after the pharmacological treatment. They consisted in cultural tests on pharyngeal swabs or on pools of faecal pellets. Once the tests resulted negative for Klebsiella spp., the mice went back to the normal routine of health check-ups, consisting of quarterly tests on sentinel animals. During the considered study period, routine tests never showed positive results for Klebsiella. This confirms that the enforced procedures - pharmacological, sanitization and working procedures - have been successful in containing bacteria. Animal welfare is a complex issue that includes also physical health. This entails a strict control over the potential diffusion of opportunistic pathogens that may cause health issues, especially in research environments where immunocompromised animals are used. A good control can only be achieved through effective and complete communication about health status among animal facilities that share animals. The compiling of comprehensive reports allows to have an overview of the animal health and therefore to recognize all manifestations that may arise in the future. This compilation perfectly fits into the "Refinement" concept, understood not only as the improvement of procedures directly aimed at the animal but also as the management of everything that regulates the life of the animal and therefore its use.

PD15 Merits for using gloves when handling Zebrafish for the collection of sperm during cryopreservation

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perm cryopreservation has become a suitable choice for conserving and archiving zebrafish (Danio rerio) strains. Although a few reliable protocols have been developed and are currently in use, very little emphasis has been placed on fish handling technique.
Sperm samples collected from males are small, typically only 0.5 -2µL, and can be lost if not collected properly so correct handling is vital for both maximizing the amount of sperm released and ensuring the fish fully recovers without injury. Methods for obtaining sperm include gentle squeezing with smooth forceps or using index and forefingers so as not to obstruct the urogenital opening and prevent any loss of sperm. While it may seem easier to control both the fish and sample collection when handling without gloves, this will increase the chance of sample contamination and can cause a higher instance of scale loss. As skin mucus acts as a barrier against infectious disease, any small amount of disruption may result in fish becoming more susceptible to pathogenic microorganisms and therefore it is crucial that handling be kept to a minimum. To identify the extent of scale loss caused by non gloved against gloved handling techniques when squeezing males, scales were counted post-abdominal pressure application and were found to be significantly higher on damp forefinger and thumb without gloves compared to that of various glove types. This emphasizes the importance of both wearing appropriate gloves and of appropriate handling technique as a refinement during the cryopreservation procedure.

PD16 Effective permethrin treatment against fur mites (Myobia musculi) on genetically modified mouse colonies housed in isolators and in Individually Ventilated cages.

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Despite the improvement of diagnostic technologies and higher sensitivity about the importance of health surveillance in rodents, fur mites infestations are frequent. Ectoparasites impede the sharing of scientific models and impact on animals' health and welfare. We investigated the effect of permethrin on a natural infestation by Myobia musculi of 24 different genetically modified mouse colonies housed in positive pressure isolators and on 6 colonies housed in IVC with disposable cages.
Materials and methods Mice: We conducted the study on a colony containing 2800 genetically modified mice including 30 different strains, housed in 9 plastic film isolators (50 cages size) and in 88 disposable individually ventilated cages (IVC). Diagnostic techniques: the contamination by Myobia musculi was detected in one isolator where 2 soiled bedding sentinel mice for every 50 cages were tested during the routinely screening via direct microscopic pelt examination after euthanasia. Due to the unique breeding requirements and the movement of active breeders, we suspected possible contamination of another eight isolators that was confirmed using the scotch tape impression test. The same techniques

were applied to detect the contamination on 6 different strains received in our quarantine station and housed in a IVC rack with disposable cages. Treatment: We used cotton balls bedding impregnated with 7.4% permethrin (Mitearrest, EcoHealth, Boston, MA). A total of 3 cotton balls per adult rodent and 1 cotton ball for each pup were placed in all cages as nesting material and replaced weekly at cage cleaning. The mice easily accepted the cotton balls using them to build nests. After 6 weeks of initial treatment we paused for a period of 2 weeks following an additional treatment for 3 weeks. Results At the end of the treatment, health monitoring was conducted on both original animals and dirty bedding sentinels via microscopic pelt examination after euthanasia. All animals remained mite-free to date (18 months, N=530 tested mice). Discussion It had been documented permethrin is effective to eradicate mite infestations in combination with an environmental decontamination. In isolators, the environmental decontamination by washing and autoclaving of cages and equipment is unpractical. We assessed therefore the treatment by using only permethrin cotton balls nesting. We compared the results obtained from mouse housed in isolators versus mice in disposable IVC cages and all were free from mites. The treatment had no adverse effect and no impact on the breeding performance of the colony, mice's behaviour or pups' vitality. Conclusions: Our findings indicate permethrin to be a reliable treatment agent when a correct length of treatment necessary to prevent re-infestation is respected. To our knowledge this study is the first which analyzes the efficacy and the feasibility of a treatment against mites on mice housed in isolators.

PD17 Nutritional supplementation using fluid diet in challenging experimental models in the primate

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Non-human primate (nHp) disease models are an important tool for the development of relevant advancements in biology and medicine. However, experimental conditions may compromise the nHp ability to spontaneously introduce the amount of food to meet their caloric needs.
Objective: The objective of this study was therefore to develop and apply a method to improve the nutritional status of animals used in challenging research. M&M: In a life supporting model of kidney xenotransplantation and in a Parkinson's disease model in nHps, we observed inadequate spontaneous food intake and a prolonged period of insufficient nutrition that may compromise the animals health status. Over the years, with the support of pediatric clinical nutrition experts, we have refined the approach to enteral nutrition in experimental nHp. In particular, we administered a hypercaloric solution in addition to the voluntary food intake to meet the nutritional needs of nHp that were at risk of significant weight loss (>20%) in a short period of time (< 1 month). Based on clinical observation and in the presence of weight loss exceeding 5-10% the administration of a hypercaloric solution was initiated. This diet is based on powder nHp feed, Nutrison Energy (a polymeric hypercaloric diet), sugar, olive oil and water, and is characterized by a nutritional value of 240 kcal/100ml. This solution was offered ad libitum (60 ml/kg/day in 2-3 times) to sucking animals or administered by enteral tube feeding (gavage) in non-collaborative nHp during the administration of the immunosuppressive therapy. Daily body weight and weekly biochemistry (including albumin) were determined. Results: nHp were monitored for an average period of 274 days (range: 7-871 days) with no animal loss due to weight loss or the onset of a wasting condition. Indeed, nHp were able to maintain their pre-study body weight and biochemical parameters remained within normal ranges. Animals were able to resume the ability to ingest food spontaneously, thus the use of gavage was limited to a short period. Whenever possible, enteral formulas were administered through spontaneous licking. Furthermore, ingredients to prepare the proposed diet are readily commercially available and cheap. nHps like the taste of the fluid diet developed and usually suck it spontaneously. Although the fluid diet was rich in energy/calories we did not observe episodes of diarrhea. Conclusion: Our study suggests that the use of an enriched dietary support such as the one described here represents a useful tool to prevent the weight loss that may occasionally occur when performing preclinical research using challenging experimental models in the primate.

PD18 The effect of noise and music upon the behaviour, stress state and corticosterone level of 1-21 days old chicks

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Noise exerts negative effect on the animals. Some chicken strains are notably sensitive to noise-related stress, but the conditioning with noise or music in young animals might have a positive effect on this sensitivity in older ages. The effect of 10-day-long music and noise exposure on the behaviour and stress state of young meat chicks has been studied, which may have practical significance.
Fifty chicks were divided into three treatments. The first group (n=17, "N") has been treated with a looped 47-minute-long mix of different noises (from 8 a. M. to 6. p. M.) during 10 hours. The second group (n=16, "M") has been exposed to the Goldberg Variations of Bach at the same time. The control group (n=17, "C") has been housed in a silent room. On the second day a study of ethograms has been carried out with each animal. The animals were studied in an empty room, where they has been placed on the open-field (OF) vivarium for 5 minutes. The weight of the animals has been measured after the OF-tests. On the 7th, 14th and 20th days another ethograms has been registered, but during these OFs a 3-minute-long uncomfortable and loud noise-mix was played. On the 14th day the animals has been placed on the OF in pairs. After each OF-test the tonic immobility time of the animals was measured, which is an indicator of the stress state of birds. On the last day of the study (21st day) blood samples has been collected from each animal to measure the serum corticosterone level and a necropsy was performed to find stress-related changes and to determine the sex of the animals. For a histopathological examination samples were collected from the jejunum, the liver, the tibia and the spleen. During the necropsy the weight of the liver, spleen and thymus was measured. To determine the fluctuating asymmetry, the length of the 3rd toes has been measured on both legs of each birds. There was no significant differences in the weight of the animals on the last day ($p>.05$). The longest immobility time was measured in the "N" group on the 1st week followed by the "M" and the "C" group, while on the 2nd and 3rd week it changed. The difference is not statistically significant. The number of tries needed to induce immobility was inconsistent and no significant differences has been found. There were no significant differences in the weight of the measured organs. The fluctuating asymmetry was

significantly higher (p<.05) in the "C" group ("N": 1.59±1.50, "M": 1.50±1.90, "C": 3.38±2.33 mm). The concentration of corticosterone was significantly lower (p<.05) in the "C" group ("N": 18.45±14.62, "M": 19.93±10.85, "C": 8.18±5.91). The histopathology did not show any stress-related changes in the birds. According to these results the music meant just noise for the poultry, but the conditioning with noise in young animals may be promising to avoid noise-induced stress in later life.

PD21 Energy monitoring and savings in the management of an animal facility.

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GSK Vaccines

Energy-related costs account for a large part of the budget of an animal facility and have a significant impact in term of ecological footprint. Various measures can significantly decrease the consumption of energy.
By energy we mean the energy required for heating, cooling, humidification, ventilation and lightning of the rooms. Energy is also highly consumed to operate the autoclaves (steam / electricity/ cooled water) and to operate the washing machines (water / electricity). An evaluation was conducted to see the biggest energy consumers and prioritize actions on them. Air change of the rooms (right temperature with appropriate humidity setting) was identified as a key consumer as well as the use of autoclaves.Various measures have been put in place to work on these aspects:- Optimization of the management of housing conditions to increase the density in the room- Application of an alternative system to correct temperature and humidity conditions - Automatic regulation of the ventilation according to ammoniac level- Adaptation of levels of ventilation for rooms without animals (offices, rest rooms located in area) - Validation of an alternative method of decontamination reducing the use of autoclaveThose steps have been implemented gradually during last year and some positive impacts are already noticeable on the energy consumption.

PD22 Implementation of an Occupational Health and Safety - tool in Research

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The objective of this project is to facilitate the implementation of an Occupational Health & Safety-tool designed for research procedures. Many sector-specific tools have been proposed and developed for risk assessment and management. In our project we tried to produce a simple tool for risk assessment in Research and test it in an ABSL-2 procedure. We tried to create certain algorithms/actions by which we can manage/control the hazards that occur during any experimentation procedure.
An overall Risk Assessment Process has been established. The core of this tool is simple algorithm guidelines and document implementation. We created three documents in order to use this tool: The "Risk Assessment" Poster, The "Risk Assessment" Form & The "Risk Assessment" Matrix. The Risk Assessment Process algorithm is outlined as follows: Select Task/define Operations=>Select Risk Assessment Team=>Set Risk Assessment Conditions=> Identify Hazards (ATAH)=>Rate likelihood & Severity=>Check Risk Rating (without Controls)=>Take Appropriate Control Measures=>Ensure Risk is ACCEPTABLE=>Ensure ALL hazards have been managed successfully/Reevaluate=>Document the Risk Assessment. To test this tool we chose an ABSL-2 procedure of Toxoplasma gondii inoculation in mice. Experienced personnel for this procedure were chosen regarding animal handling and previous experience in animal inoculations. Immunocompromised persons and T. gondii -seronegative women who are pregnant or might become pregnant should be counseled about the risks associated with T. gondii infection. We set that this procedure would be conducted in an ABSL-2 laboratory regarding the risk level of the inoculating agent. Hazards emerging from the procedure and the agents used were identified and classified in categories. T. gondii is classified as a risk level 2 biological agent. Biological containment Level 2 facilities, equipment along with operational practices for work involving infectious or potentially infectious materials and animals were used and followed. Hazards identified were spills, sharp objects, disposal, storage, mice bites, feces from infected animals. Parenteral inoculation, exposure to mucous membrane or skin lesions (wound), ingestion, or aerosols were considered as hazards. Changing litter, cages, instruments and glassware were considered contaminated and were sterilized. Sterilization was considered as the most appropriate way for decontamination. Appropriate PPE was chosen regarding the appropriate containment requirements occurring from the agents and the procedures used.

PD24 Evaluating sanitation effectiveness in isolator: a simple protocol.

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The Guide For the Care and Use of Laboratory Animals states that solid bottom caging requires sanitation at least once at week and underlines that regular evaluation of sanitation effectiveness is recommended. In isolators, cleaning and disinfection of cages surfaces and accessories is realized in a closed environment where animals are living. We developed a simple protocol to evaluate sanitation effectiveness in this special piece of equipment.
Materials and methods. We conducted the study selecting randomly 10 cages from a plastic film isolator set on positive pressure to be sampled before and after the standard sanitation procedure. The selected cages were solid bottom plastic cages made in polypropylene, housing a number of adult mice C57BL/6JOlaHsd of different gender ranging from a minimum of 3 to a maximum of 7. We assessed the effectiveness of sanitation monitoring using a physical parameter or a microbiological parameter on 5 different cages. The physical parameter assessment was done by visual inspection to evaluate the effective elimination of a test soiling introduced in the bottom surface of the cage immediately after removing dirty bedding material. The test soiling was realized using 20ml of boiling instant coffee left in the cages for 15 minutes to produce a dirty slide. For the microbiological test colony forming units (CFU) on rodac plates were counted pre and post disinfection. The cages were cleaned and disinfected using ethanol 90% v/v (contact time 1 minute) and well wiped with irradiated disposable soft paper. Results The acceptance criteria were the following, a cage was considered "sanitized" if there was a complete elimination of coffee residuals by visual inspection and a reduction of 50% of colony forming units (CFU) post-disinfection was observed. At the end of the disinfection procedure all the cages were visually completely clean and reduction of 60-70% of CFU was observed in all the tested cages. Discussion. Sanitation involves cleaning which removes excessive amounts of excrement, dirt and debris, and disinfection reducing or eliminating unacceptable concentration of microorganisms. Methods of solid bottom

caging sanitation are usually based on washing and autoclaving, while these procedures are not easily applicable in isolators populated with animals. Sanitation of cages in isolators can be unpractical and assessing the effectiveness can be difficult to realize, but this aspect is important for the maintenance of environmental conditions conducive to health and well being of animals. We assessed therefore a simple and reproducible protocol. Conclusions. Our findings indicate that ethanol 90% v/v is a reliable agent to guarantee the correct sanitation of cages in isolator. Moreover, ethanol is a cheap agent with no adverse effects on operators and animals and on equipment components and materials.

PD25 Noah's Ark in Montreal: Consolidation and modernization of the animal facilities at the Research Institute of the McGill University Health Centre.

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The presentation will be a detailed overview of the different steps and challenges facing an Animal Resources department during the move and consolidation of their 6 animal facilities across the Island of Montreal (12 000 rodent cages) into two facilities. Key topics to be discussed are: 1) animal transfer/rederivation 2) equipment procurement and placement and 3) Human Resources reorganization.
At the start of 2015, the Research Institute of the McGill University Health Centre (RI-MUHC) finalized their redevelopment project by conducting a major move to consolidate its laboratories and animal facilities. The Animal Resources Division (ARD) centralized its operations, previously spread over six sites, to two locations: the Montreal General Hospital (MGH) and the newly built Glen site. The Glen vivarium is a 5,000 square meter facility. It includes the following state-of-the art sectors: Biosafety Level 2, Biosafety Level 3, Quarantine, Behavioral, Surgery and Imaging. The facility has housing units for up to 15,000 mice cages with flexibility to house rats, rabbits, guinea pigs, chinchillas and ferrets. Together, both MGH and Glen animal facilities offer services to 110 Principal Investigators (PI) with 213 active animal use protocols. A significant amount of planning by the ARD Management was needed for the transition, most notably for: 1) animal transfer 2) equipment placement and 3) Human Resources reorganization. Over 90 transgenic mice strains had to be cryopreserved and rederived as the health status was substandard in many of the predecessor facilities. Ultimately, 3000 rodent cages were physically moved over a 3-day period. The procurement and eventual reception of a significant amount of preclinical research equipment needed to be budgeted for and purchased, often via public tender. As the ARD employs 40 staff, several strategies were necessary to restructure its organization. Decisiveness, persistence and a willingness to adapt were essential to the success of a move of this magnitude where both animal welfare and updating research objectives were essential guiding principles.

PD26 Care For The Carer? An ACT Health Initiative.

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Animal carers are required, as a part of their daily routine, to care for sick animals or to cull animals that cannot be used in experiments. Some animals may be young, others old and others sick but the latter makes the task no less stressful As very caring people who do not often have the chance to discuss their work outside of their immediate and understanding environment, they experience sadness and can be become depressed, especially if they are asked to cull many animals in one go.
While many staff develop coping strategies, including but not limited to the ability to reduce the stress load by splitting the cull across a few days, reading references and reviews on coping methods, training at university, memorial services and of course good team support, there are still staff who do not cope as well as others. Key issues identified have been:

- A need to debrief
- A need to share the load
- A requirement for time out following and/or prior to a large cull
- Acknowledgment of their compassionate natures by the researcher
- A need to learn how to let go

In ACT Health a group has been established to assist staff from all areas across the division with coping strategies. While it is not unusual for a division of health to support the mental health of its health workers such as palliative care, aged care and paediatric staff, it is unusual for the group to include animal care staff. ACT Health has acknowledged that there are common issues across these areas and are endeavouring to provide this support. In addition, we have created a survey, which will be sent to ANZLAA members with the aim of preventing or reducing incidence and establishing support groups for those affected. This presentation will provide a status update on this working group and will highlight core outcomes, which should be considered by facility managers in managing the mental health of their staff.

PD27 Prevalence of Infectious Agents in Pet Mice

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The objective of this study was twofold: first, to better understand the risk associated with pet mice possession for animal caretakers, and second, to use the data as training and awareness for employees (1).
Materiel and methods: 10 mice were purchased in 5 different pet shops within a 30 km radius of the Charles River Les Oncins site (France). The chosen pet shops were located in the cities representing employees' most common living areas. Mice were chosen between 5 and 12 weeks old. The tests performed were a comprehensive health monitoring (bacteriology, serology, parasitology, PCR on individual animals and PRIA surveillance plus from pooled animals [feces/oral swabs plus fur swabs] from the same pet shop). Results: The mice were found to have a variety of infectious agents: 10 viruses, 9 pathogenic bacteria and 10 pathogenic parasites. Among those agents, some were zoonotic organisms such as Hymenolepis nana, Giardia sp, Cryptosporidium sp and Campylobacter sp. Several viruses were found, including three parvovirus subspecies (MVM, MPV2, MPV3) which are the agents most often detected in animal facility contaminations. The bacteria found were relatively common, and some of them are considered as opportunistic. The most surprising finding was the high prevalence of parasites, especially Hymenolepis nana,

Polyplax serrata and Giardia sp. Conclusion: The study confirms that maintaining pet mice at home can expose people to many germs. Most of the germs detected in pet mice are in the FELASA exclusion list. People that have pet mice or rats at home can be potential germ carriers (via clothes, shoes, hands, saliva and skin), which increases the risk of contamination in an animal facility.

PD28 Microbiological status in relation to clinical symptoms and vice versa

de Bruin, Wieke and Van de Ven, Esther Presenting authors.
QM Diagnostics.

In general, presence of infections in an animal colony are not immediately obvious as the majority of pathogens do not cause overt clinical disease. Immune deficient animals are an exception as they become ill when infected with certain pathogens, whereas immune competent animals seem healthy. In addition, the presence of clinical signs or illness may be due to other factors than a pathogenic agent.
One should not be surprised that infections are found without any clinical signs in the animal population. On the other hand, one should be alerted that an infection may be involved at changes in the welfare of the animals or when sudden deviations in research outcome are found. This presentation will show some examples of clinical findings and the causative pathogens.

PD29 A prerequisite for maintaining microbiological barriers

de Bruin, Wieke and Van de Ven, Esther Presenting authors.
QM Diagnostics.

Hygiene and a good cleaning regime are the prerequisites to reduce the risk of introducing unwanted infections into the animal facility. What may seem optical clean is not always necessarily microbiological clean. Everything which is introduced into the animal facility (consumables, disposables, equipment, etc) needs to be considered a potential hazardous risk.
A short review is provided of measurements animal laboratory facilities can take to reduce this risk of introduction and spread of infections. This presentation will focus on disinfection, sterilization and cleaning procedures and why it is important to validate autoclaves, spray-, transfer- and air locks and cleaning procedures to ensure their quality.

PD30 Methods and samples of choice for reliable health monitoring

de Bruin, Wieke and Van de Ven, Esther Presenting authors.
QM Diagnostics.

Simple reliable and cost effective health monitoring (HM) is a desire of every animal facility manager. The introduction of Individually Ventilated Cages (IVC) has not made HM easier. Detection of infections is quite difficult as every cage is a microbiological unit and cage to cage transmission of infection as happens in conventional housing systems is reduced to almost zero.
Alternatives to reduce the use of sentinels like life-saving sampling of colony animals in the rack or testing the IVC rack filters and plenum are currently under investigation. As expected each approach has its advantages and limitations and there is not one simple solution for HM. This presentation will give an overview of these possibilities and restrictions.

PD32 Does conventional housing and different types of bedding materials affect the ethology and welfare of laboratory animals?

Vijayakumar Sreelatha, Harikrishnan, Presenting author.
SCTIMST.

This work compares the impact of conventional housing and barrier housing set-ups on the welfare and ethology between laboratory rodents. The study was conducted in 48 adult Wistar Rats and 48 Swiss Albino mice of both sexes where fecal corticosteroid assays and ethological tests were employed to demonstrate whether housing the animals as well as routine management procedures has any added impact on conventional housing when in comparison to the rodents housed in barrier maintained facility.
Easy to operate, less expensive conventional facilities still prevails among a larger section of laboratory animal facilities in developing countries. Historic buildings, lack of funds and lower incidences and reporting of major disease outbreaks and un-revealed sub-clinical infestations that still lie concealed from getting into the notice of facility managers and laboratory animal veterinarians has caused a slowdown in the pace of upgrading the facilities. It is known that the health status differs between conventional and barrier types of housing. But studies demonstrating the effects of conventional housing on welfare and normal behavioural repertoire are required to be documented to learn and understand about the specific advantages or disadvantages it should have while we compare it to the advanced state of art housing under controlled environment and in individually ventilated cages. Traditionally paddy husk is being used widely in India as bedding material and it has several disadvantages like reduced absorbency eventhough it has an advantage of low cost and easy availability. The effects of paddy husk bedding on animal welfare and behaviour had not yet been studied and this work focuses to this point as well. Nest building tests in mice and behavioral anhedonia using 1.5% w/v sucrose as well as faecal corticosteroid assay is used to demonstrate and compare the effects of conventional housing and barrier housing in individually ventilated cages on welfare and behaviour in Wistar rats and Swiss Albino mice.

PD33 The Fourth R - Redefining the Three R's in a Rodent Health Monitoring Program.

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1. Reevaluate your rodent health monitoring program at least annually, and adjust parameters based on the size and specific needs of your animal care and use program to minimize the number of animals required.
2. Review and incorporate new technology to increase the scope and sensitivity of surveillance to replace animal usage.
3. Recalculate the cost/benefit ratio of use of the new technology, and review the periodicity or frequency of testing to further reduce costs and the number of animals required.

By applying these principals to our health monitoring program, we dramatically reduced animal usage by over 1/3 (8,000 mice vs 5,000) (600 rats vs 350) annually. Incorporation of PCR technology increased the sensitivity and comprehensiveness our surveillance program, by submitting pooled samples colony animals or sentinel animals on an annual basis. In the first year, we discovered both fur mites (Radfordia myobia) and pinworms (Aspicularis tetraptera) in two rooms in different facilities. Successful treatment and subsequent eradication was confirmed with PCR technology. The use of PCR technology is especially critical for our very large program, because we retain the ability to test all vivaria in a short time frame, to capture a "snapshot" of all facilities and campuses. This important element allows for expeditious treatment of "hot spots" and decreases the likelihood of transfer of agents between facilities. Consequently, as a result of refined detection techniques, including use of PCR technology to screen imported animals in quarantine, we have changed the periodicity of testing from quarterly to triennially. This has resulted in a dramatic reduction in the number of animals used for surveillance, as well as reduced costs for testing. Additionally, further review of new data published by IDEXX1, clearly shows that animals up to eight months of age are capable of strong serologic responses to antigens. We have incorporated this parameter into our program by retaining the oldest cohort sentinel for annual testing, thus reducing the number of replacement animals ordered after each testing period by half, yet increasing our ability to detect pathogens. The cost savings gained from reduced purchase of animals and fewer per diem days, in addition to reducing the frequency of testing, exceeded the cost of PCR testing. The cost savings for labor involved (reduced frequency of testing and reduced amount of in house testing for parasites) was equivalent to a 0.5 full-time veterinary technician position at our institution. To Redefine a rodent health monitoring program Reevaluate your rodent health monitoring program at least annually to reduce animal usage; review and incorporate new technology to increase the scope and sensitivity of surveillance to replace animal usage; and recalculate the cost/benefit ratio of use of the new technology, and review the periodicity or frequency of testing to further reduce costs and the number of animals required.

PD34 Germ free environment is not changing reproduction capacity of the IL10-deficient mice, a mouse model of inflammatory bowel disease

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Germ free (GF) mice are devoid of any other living organisms than their own cells and their maintenance requires a profound expertise. The IL10-deficient mouse model is a good model for studying inflammatory bowel disease (IBD), as disease onset is influenced by mouse genetics and intestinal flora. Under GF conditions these mice do not develop colitis. However, GF housing can influence their reproduction. The study aimed to analyze the breeding efficiency of GF IL10-deficient mice.
C3Bir.129P2/JZtm-Il10tm1Cgn (C3H-Il10-/-) breeding females, which are more susceptible to develop severe colitis and B6.129P2/JZtm-Il10tm1Cgn (B6-Il10-/-) breeding females more resistant for developing colitis were monitored over one year under germ free, conventional and specified pathogen free conditions (SPF). From collected data number of litters, born and weaned pups were determined. Furthermore, intestinal histology was analyzed in an additional cohort of IL10-deficient mice.No difference in number of born pups per litter was determined in mice housed under GF, SPF and conventional conditions. The average number of born pups per litter was around 5. Additionally, no difference in number of born pups was observed between C3H-Il10-/- and B6-Il10-/-mice in all housing conditions. Number of weaned mice also did not differ among two strains and housing conditions and was around 4 weaned pups per litter. However, the number of departed pups prior weaning was lower in mice housed under GF conditions. Furthermore, females of both strains showed tendency to have more litters when held under specified pathogen free or GF conditions. Moreover, histology analysis of intestinal samples revealed no signs of intestinal inflammation in GF mice. SPF housed mice showed a mild gut inflammation and conventionally housed mice displayed even higher histopathological scores. Even due to biochemical and physiological adaptation to the life in a sterile environment, the reproduction capacity of IL10-deficient mice stays stabile. The average number of born and weaned pups is comparable with the mice born and weaned under SPF and conventional housing conditions. However, the number of deceased pups prior weaning is higher in regular housing conditions than in GF conditions, probably due to exposure to environmental germs. Additionally, females housed in GF and SPF conditions give more litters than conventionally housed mice. This correlates with the histopathological score, and the reduction in litter numbers may occur due to colitis onset. Altogether we showed that GF IL10-deficient mice display a good reproduction ability which is similar to mice reared in regular housing conditions.

PD35 Highly Sensitive ELISA for the Serological Detection of Murine Rotavirus EDIM Based on its Major Immunogen VP6

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Health monitoring of laboratory animals is a critical factor for the surveillance and validation of animal experiments. Rotavirus EDIM is a highly infectious agent with an estimated prevalence of 1.7% to 3.7% in Western Europe and can interfere with research. In our serum database, EDIM occurred with a prevalence of 0.24% (8 of 3243). To test whether this is attributed to the low sensitivity of the applied test or a low prevalence in Germany, we developed a highly sensitive VP6-based ELISA.
Sera of BALB/c and B6 mice nasally infected with EDIM were tested with a commercial ELISA and a virus-based ELISA. Sera with deviating results (n=15), were tested by an immunofluorescence assay (IFA). Western blots were used to identify the main epitopes of EDIM. Identity of the viral proteins was confirmed after SDS-PAGE by analyzing the tryptic digests by nanoRP-HPLC-QqTOF-MS. The major immunogen was expressed in E. coli with Strep- and His-tags and purified by IMAC. The protein was used as ELISA antigen and validated with negative sera and sera positive against EDIM, MNV, MPV, MVM, MRV, and P. pneumotropica. The commercial ELISA detected the infection in 14 of 29 sera of EDIM-infected mice (sensitivity 48%, n=29; specificity >99%, n=376), whereas the virus-based ELISA identified all serum samples as positive (sensitivity >99%, n=29) without false-positives (specificity >99%, n=200). Differences in the seroconversion of BALB/c and B6 mice were not observed. Identification of immunogenic proteins relied on a virus protein preparation and all structural viral proteins and three of the six non-structural proteins were identified. Immunoblots with EDIM-positive sera revealed VP6 as the main immunogenic protein. Next, tagged VP6-protein (Strep-rVP6-His) was expressed in E. coli, solubilized from inclusion bodies, and purified by IMAC providing high purities and protein yields of over 90 mg/L culture. Protein identity and immunogenic properties were confirmed by SDS-PAGE, MS, and western blot. An indirect ELISA using Strep-rVP6-His showed a

sensitivity and specificity of >99% (n=29 and n=376) and a selectivity of >99% against multiple FELASA-pathogens (MNV: n=77, MPV n=10, MVM: n=4, MRV: n=5 and P. pneumotropica: n=18).In this study, a Strep-rVP6-His-based ELISA for the serological detection of EDIM infections was developed. The ELISA was applied to serum samples from deliberately infected mice and of mice from animal facilities in Germany. The test required less than 3 µL of serum enabling the possibility to collect blood from mice while keeping the animal alive. Moreover, the Strep-rVP6-His-based ELISA showed significant advances compared to other commercial tests. Undetected EDIM-infections support spreading of the disease and can influence research, as shown by Collins et al. and others. Therefore, this work contributes to an improvement of health monitoring of EDIM and, thus, to animal welfare. B6: C57 black 6; EDIM: epidemic diarrhea of infant mice.

PD36 Lack of transmission of murine norovirus by assisted reproductive technologies.

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INTRODUCTION: Since its discovery in 2003, murine norovirus (MNV) is still endemic in many rodent animal facilities. Our aim was to determine the risk of transmission of MNV to recipients and pups via assisted reproductive technologies (ARTs), especially those which compromise the integrity of the zona pellucida (ZP).
MATERIALS AND METHODS: Immunocompetent C57BL/6NTacCnrm (B6N) and immunodeficient athymic nude (nu/nu) mice were naturally infected with MNV (91% homology to MNV3). In vitro fertilization (IVF), assisted IVF with reduced glutathione (AIVF), intracytoplasmic sperm injection (ICSI), and ovary transplantation were performed with these mice. The resulting embryos were transferred to 0.5-dpc pseudopregnant CD-1 females. Half ovaries from B6N and nu/nu mice were transplanted into B6N or nu/+ recipients, respectively. To detect MNV in spermatozoa, embryos, recipients and pups, qualitative and quantitative RT-PCRs were performed. Recipients and pups were also analysed via serology. RESULTS: The mesenteric lymph nodes, small intestine, spleen, liver, lung, brain, ovary and testis were infected at specific intervals over a one-year period. The peak infection of the sex organs was at 12 weeks with 20 to 50 viral genomes/mg gonad. MNV strictly adhered to spermatozoa collected from infected mice since three washes did not remove MNV from the sperm. After using MNV-positive sperm for IVF, AIVF and ICSI, 27 to 30 genomes were detected in IVF (n = 100) and AIVF (n = 100) embryos from both mouse strains. Approximately 87% of MNV detected in these embryos was found in the ZP. All embryo transfer recipients, pups and ovary recipients were MNV-negative. CONCLUSION: The results indicate that manipulation of the germplasm through ARTs did not lead to transmission of MNV to mice. This may be due to the absence of an infectious dose or failure of the MNV strain to replicate effectively in developing embryos and the reproductive tract.

PD37 Rodent caging system and associated environmental control, OHS, facility and veterinary management issues: a review of past and current solutions and practices, ongoing evolutions.

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The history or rodent caging and care in animal-based research went through many evolutions and innovations. Animal welfare standards, Quality and good research practices, the ever increasing variety and sensitivity of rodent models… and the related environmental and health control requirements led to more and more elaborated caging concepts accompanied by a continuous evolution of facility, OHS and veterinary management
Over the last 50 years, rodent-based research was in constant evolution: animal welfare / 3Rs regulations & standards; Quality; good research practices; increasing number, variety and sensitivity of rodent models (incl. highly sensitive or immunocompromised models); emerging need for combined bio-containment and bio-exclusion or microflora control… This led to more stringent environmental & health control requirements, leading to more sophisticated caging and housing concepts such as Individually Ventilated Cages (IVC) systems and IVC Biocontainment Units (BCU), responding to many other expectations: optimization of capital investment; animal facility capacity / versatility; micro- and macro-environment monitoring / records; facility management (e.g. administration, cost control, cage census); OHS requirements (animal allergen control, ergonomics); health monitoring, veterinary management (response to behavioral needs, cage checks / records…). Veterinary management issues include a response to health monitoring challenges, due to the sky-rocketing increase first of static filter-top cages (FTC) and IVC systems; to the increased and variable complexity / sensitivity of rodent models; and the multiplication of animal origin and health standards. In parallel, veterinarians had to deal with the identification of new rodent infectious or parasitic agents (either pathogenic, opportunistic or interfering) and new techniques of health screening, diagnostic techniques and sampling strategies. Two examples technical advances are worth being more thoroughly addressed: (i) in IVC health monitoring, the replacement of animal sentinel use by reliable and validated PCR-based approaches, and (ii) in cage environmental control (open cages, FTC's, IVC's, BCU's), the monitoring of temperature, humidity, C02 and NH3 air concentration, first at room level, then at each IVC cage level. Environmental monitoring benefits from modern tools and technology (Ipad, Wifi / Bluetooth connections) allowing continuous, central and remote control and recording of these key environmental parameters, as well as a continuous control of technical defaults (temperature, ventilators, filters, electricity supply), generating 24H/24H and 7D/7D alarms and records, while reducing associated manpower. Further to rodent individual electronic identification, radio frequency identification (RFID) cage tags and readers (mobile or room dedicated) can now be used for computer-assisted cage census.

PD38 Exhaust Air Particles (EAP) PCR marks a change in paradigm in health monitoring of IVC housed mouse colonies.

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Animal health and hygiene status are central for standardization and reproducibility of rodent studies. Individually ventilated cages (IVC) have become the predominant rodent housing system as they prevent aerogenic pathogen transmission. The major drawback of IVC systems in view of health monitoring is that sentinel animals exposed to soiled bedding (SBS) no longer detect many infectious agents.
A novel method uses exhaust air particles (EAP) analysis instead of SBS. The idea of using nucleic acids associated with particles in the exhaust air of IVC cage rack systems for microbiological monitoring was published by the Helmholtz Zentrum München in 2009 (EP 2 103 208 A1). Since then intensive research was conducted at the Zentrum with the aim of identifying the materials and the most reliable methods to be applied to envi-

ronmental microbiological monitoring of IVCs. EAP real-time PCR was systematically compared to conventional SBS monitoring for 4 commonly found viral and bacterial mouse pathogens. EAP samples detected the pathogens reliably, while SBS failed to detect infected animals in almost all instances. The EAP method proved highly sensitive and the minimum prevalence for a positive result was commonly one cage with infected mice in a 63 cage IVC system. Pros and cons of the new technology will be discussed.

PD39 Current knowledge and challenges to fulfil nutritional requirements in cephalopods

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Cephalopods are all carnivore predators known to rely on live preys. Their dietary breadth spans from generalist/opportunistic to specialist depending on the species (around 700). Cephalopods as laboratory animals are now regulated by Directive 2010/63/EU. Proper feeding regimes and prey choices should be established to fulfil care and welfare requirements. Best-practices should also consider ethics, health monitoring and requirements for standardization in laboratory practice.
METHODOLOGY - STATE OF THE ART Apart from very limited exceptions, Cephalopods are all marine carnivore predators known to rely on live preys. Knowledge on cephalopods diets is based on different studies coming from laboratory research, fishery and aquaculture. Laboratory feeding regimes are linked to best practices approach and availability of potential prey species. In addition, standard conditions for feeding cephalopods at different life-stages are not always completely understood, and information derived from other vertebrates do not necessarily apply to these species due to their uniqueness (e.g. physiology, higher metabolic rate, different lipid metabolism and importance of polyunsaturated fatty acids). Requirements for cephalopods feeding have been compared in aquaculture and laboratory research. For example, according to literature (scientific papers from 2010 and 2013) many european laboratories provide daily food ad libitum, mostly with live species (e.g. mysids, shrimps and crabs) or frozen alternatives, mostly fish and shrimps. In aquaculture, instead, effort has been carried out to implement artificial diets by pellet formulation (e.g. for the common cuttlefish). Main drawbacks of live prey system include the use of other live animals (mainly crustaceans, that have to be treated ethically and legally), their possible microbiological contamination, appropriate removal of excess food and food debris. Artificial diets can overcome these problems, but should be palatable, with proper presentation and providing all nutritional requirements. However, other aspects need to be considered when establishing feeding protocols (diet and frequency): water temperature, life stages, food quality. RESULTS AND DISCUSSION We will list cases and overview recent and classic literature including information on the nutritional physiology of some species, thus providing the ground for establishing optimal suggested nutritional requirements for the most commonly used cephalopod species.Despite live-prey feeding would be fundamental for keeping natural cephalopods behaviour, alternatives should be developed to fulfil ethical and health requirements. Research in this area is still at its infancy, but use of adeguate alternatives and possible pellet formulation should be encouraged in laboratory practices, keeping fulfilment of all nutritional requirements. This abstract is contributed on behalf of 47 members of the COST Action FA1301 "CephsInAction".

PD40 Increase animal facility control - please have your alarms in the smartphone

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The most important requirement in the animal facility is animal welfare, which is imperative to have reliable results. This study aimed real time and continuous identification and monitorization of vital operational parameters in animal facilities.
At first ambient parameters were identified. Critical requests for animal facilities: vibration, light, noise, the day/night shifts, temperature and humidity; were identified. The possibility to track environmental parameters, assure the correct flow of people and animals, equipment and sample storage devices were also identified as necessities. Air quality, in euthanasia and surgery room, were also recognized as health and safety issues. The solution proposed was a software development using a SCADA platform to assure the robustness of the system to work 24 hours 7/7 day without fails, and available in a friendly environment for computers, tablets and smartphones. The application was laboratory tested before its implementation in animal facilities, our work allowed the development of a flexible system that can integrate existing equipment, be installed in current facilities and adjusted to new requirements. The system relies on sensors, connected by wireless or cables, with a standard protocol that monitors a wide range of parameters. The system has been used for monitoring temperature, humidity and to control autonomously the intensity of the lights to have the complete simulation of day and night. The installed systems allowed the users to receive alarms when the parameters were outside of the range expected. The system sends an email, SMS or visual/acoustic alarm if abnormal parameter are detected. In this presentation some practical examples are showed. The most common result from its use was the rapid detection of temperature changes in rooms and equipment. In conclusion the system may promote animal welfare because it allows to fast track and ensure parameters in animal facilities. Due to its modular possibilities, it may also be used to increase workers safety and equipment constant monitoring.

PD41 Preliminary study of ergonomic and functional enhancements for pathology benches development

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Necropsy studies are needed in laboratory animals sciences. This study aimed the identification of ergonomic and functional needs of pathology benches, followed by the design of a bench in accordance with the requirements for good practices.
At first, information on the characteristics of currently available benches and accessories in the market were collected. Secondly, interviews were conducted to survey the concerns and difficulties to technicians (n = 4) and veterinary anatomic-pathologists (n = 10), as well as visits to laboratories in the North of Portugal. The results suggest: 1) risks resulting from exposure to fixing agents as a consequence of poor exhaustion; 2) traceability difficulties of biological material; 3) the need for auxiliary equipment for macroscopic examination, such as scale and camera; 4) lack of user-friendly technologies for data recording; and 5) complexity in the organization and access of records. It was, however, not possible to establish order of magnitude of the results presented above. This study found that the perceived needs are according to some already described. The survey conducted stressed the need to create products with integrated solutions. Thus, a pathology bench was designed with ergonomic and functional aspects optimized. The pathology bench include: 1) double filters, enhanced exhaust; 2) an interactive clean surface with suitable software to record the observations, information management and traceability; 3) integration of auxiliary equipment, without the need of bringing accessories inside the laboratory. The workbench was developed with the support of pathology technicians and with their inputs, the necessary equipment were integrated in the workbench. So it has integrated taps driven by foot operation for water and formaldehyde. In addition in the development it was integrated a dispenser system for gloves and paper for better access to these resources. In terms of software and touch interactive interface, we developed appropriate software for the activities of pathological anatomy using webcams for video, photo and audio recording, allowing a technician to record the data during the procedure. Thus this workbench integrates all the components necessary to work in this area allowing the user to read the procedure, use the necessary materials and record the data in safe conditions.

PD42 Effects Of Foster Mother On Behavior And Physical Development Of Rat Pups.

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Like human infants, newborn rat pups need maternal care for balancing their body heat and nutrition. The pups, which lose their mother for some reasons, or the first bred pups in transgenic breeding colonies are given to foster mothers. Later these pups can be used in scientific research studies. Our purpose is to evaluate if using foster mothers in breeding colonies effects the physiological development and/or pups' behavior parameters.
Material and methods: Wistar Hannover rats were used in this study. Females that are in proestrus period were mated. The pregnant dames (n=6) and their pups (n=74) were included to the study. When the pups are 4 days old, foster mother group pups (n=37) are given to foster mother while control group pups (n=37) stayed with their biological mothers. The physiological development of the pups was evaluated by their weight gain and the days of eruption of incisors, eye opening and pineal detachment. The anxiety-like behavior of the pups was evaluated with elevated plus maze (EPM) and open field (OF) tests. Results: The three physical feature, eruption of incisors, eye opening and pineal detachment, maturation parameters were not affected by foster mother care. After weaning, the weights of pups reared by foster mothers, were significantly higher than that of control group. There were no significant difference between groups in open arm and closed arm entries, and times spend in these arms in EPM. There was also no significant difference between groups in locomotor activity in OF test. Discussion and conclusion: Our results indicate that pups reared by foster mothers have gained body weight faster than pups reared by their biological mothers. Gomez-Sarrano et al. demonstrated that speed of body weight gain of foster reared pups differ according to their strains. Although effect of foster breeding on body weight is not known, Gomez-Sarrano et al. suggested that nongenomic factors are involved. Although, foster breeding affects body weight gain, it does not cause anxiety-like behavior. Foster mothers should continue to save lives of pups that lost their mothers in breeding colony. Keywords: Rat, foster-mother, anxiety like behavior, maternal behaviour.

PD43 Evaluation of breeding efficiency and welfare of mice housed at 28°C compared to room temperature under SPF conditions.

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For a constant body temperature, mammals need to balance their heat exchange. For small rodents environmental heat loss is a major problem due to their unfavorable body to surface ratio. Mice housed at room temperature are permanently exposed to cold environment because their thermoneutral zone is at [30]°C. The required energy for the essential thermogenesis probably influences the breeding outcome of mice. Hence, we were interested if the breeding efficiency for mice living at [28]°C, is higher.
Materials and Methods: Ten one-to-one-breeding cages were set up at room temperature (23°C, control group) on the one side and at 28°C on the other side. C57Bl/6J as the common laboratory strain aged between eleven and fifteen weeks were used. Twenty females and males from our in-house inbred breeding were randomly paired. Each breeding pair was fed with 280g standard chow and 250ml water. Each week mice and food were weighed and the water volume remaining was measured. Mice were kept in IVC cages for a better control of temperature and humidity. Results: The mice at 28°C consumed approximately 10g more per week compared to the control group. The water consumption for a week was comparative for both groups. We found a different nesting behavior for the mice at 28°C. Their nests were less distinctive especially when there were no pups inside the cage. The litter quantity after six weeks was the same in both groups. There was no significant difference between the litter sizes of both groups. Discussion: Mice in normal laboratory conditions are held under perpetual cold stress, as they need more food to compensate the heat loss (heat increment of feeding) [1]. As expected mice housed at room temperature showed a higher food intake but the weight of both groups at 28°C and room temperature were approximately the same. The almost equal water consumption suggests that mice at 28°C are not held under warm stress. Interestingly, the litter quantity did not differ and the litter size was not significant higher at 28°C. Conclusion: Due to the less food intake and the different nest-building behavior of the mice at 28°C compared to the control group we conclude that the mice

held nearer their thermoneutral zone are housed under more comfortable conditions. Certainly the equal litter quantity for both groups leads us to the assumption that mice at room temperature compensate their inadequate housing temperature with a higher food intake. Hence the impact of temperature on breeding efficiency is negligible but not on animal welfare.

PD44 21st century technology can enhance husbandry practices addressing the stink of ammonia, even without standards for acceptable levels.

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While a simple molecule, NH3 or ammonia is a stinky and complicated problem in lab animal management. Various husbandry processes have been implemented to delay its buildup in cage environments and avoid murine respiratory tract pathology. As an airborne contaminant, it is a challenge to measure. The "solution to this pollution" appears simply to be to detect and prevent moisture from accumulating as a substrate, which would otherwise enable the bacterial production of this harmful vapor.
Ammonia (NH3) is a colorless, "heavy", acrid-smelling gas at room temperature and normal atmospheric pressure. In the vivarium, it is familiar to us for many beneficial purposes, including quaternary ammonium cleaners and a source of nitrogen in feedstuffs. We've known too of the fundamental health issues occurring while managing mouse colonies, when ammonia forms within a wet cage due to anaerobic bacteria converting urea in the urine. And this is what stinks about it. Ammonia is a problem, capable of causing pathology to the murine respiratory tract and irritating the eyes, skin, and respiratory systems of animal care providers. Though the human nose is capable of detecting levels as low as 2 parts per million (ppm), the US Occupational Safety and Health Administration has set a higher limit of 50 ppm for persons in an 8-hour workday. For mice, living in their environment 24 hrs/day, there is little agreement within the lab animal community about what might be a standard. Some suggest cage concentrations less than 50 ppm; others believe that as a burrowing species, mice tolerate higher levels. The resolution is recognized as being multi-faceted: 1) Few systematic studies have been published to understand well the impact of ammonia in a lab rodent's world; 2) Measuring ammonia in static cages is done, but is known to be variable and presently impractical in ventilated systems; and 3) Complicating accurate detection is the presence of other airborne contaminants in both micro- and macro-caging environments. Thus, the "solution to this pollution" has tended to follow statements like in the ILAR Guide: "Bedding is used to absorb moisture, minimize the growth of microorganisms, and dilute and limit animals' contact with excreta." Management practices are designed to minimize both ammonia production and concentration, including cage change frequency, choice of bedding substrate, and cage ventilation to remove moisture accumulation. The seminar will place a call to animal health care providers to investigate and establish methods and standards for acceptable intra-cage levels of ammonia. Meanwhile, technology has emerged which focuses on means to preempt and prevent ammonia generation.

PD45 Evaluation of Exhaust Air Debris from Two Types of Individually Ventilated Cage Racks for Health Monitoring of Laboratory Mice

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The use of sentinel mice, housed on dirty bedding from colony mice, has historically been a cost-effective way of monitoring for the presence of rodent pathogens in mouse colonies. With the advent of individually ventilated caging (IVC) systems, sampling at the rack or plenum level has been proposed as an alternative to sentinels. However, no studies have comprehensively evaluated the effectiveness of rack level or plenum testing for detection of a broad range of viruses, bacteria and parasites.
In this study, we examined the effectiveness of rack level monitoring by testing exhaust air debris from two types of IVC racks for detection of infectious agents in laboratory mice. One rack design had unfiltered air flow from within the cage to the air exhaust pathway. The other rack design had a filter between the cage and the air exhaust pathway in the rack. For 12 weeks, racks were populated with either 5 cages of mice or a single cage of mice, 3 mice per cage, infected with one of the following murine pathogens: Murine norovirus (MNV), Mouse parvovirus (MPV), Mouse hepatitis virus (MHV), Helicobacter species, Pasteurella pneumotropica, pinworms, Entamoeba muris, Tritrichomonas muris, and fur mites. Shedding of pathogens by infected mice was monitored at regular intervals throughout the study. In the rack design with a filter between the cage and the rack exhaust system, testing of swabs from exhaust plenums by PCR yielded negative results for all pathogens at all time points of the study. In the rack design with open air flow from within the cage to the rack exhaust system, pathogens detected by PCR in exhaust debris included MHV, Helicobacter spp., P. pneumotropica, pinworms, enteric protozoa and fur mites. These pathogens were detected in exhaust debris from open air flow racks housing either 5 cages or a single cage of infected mice. Neither MPV nor MNV were detected in exhaust debris even though mice were documented to shed virus for prolonged periods. These results demonstrated that testing of rack exhaust air debris from racks with open air flow design was useful for detection of MHV, enteric bacteria and parasites and fur mites. However, this method of monitoring failed to reliably detect MNV or MPV infection of colony animals. In conclusion, the type of ventilated rack and the specific design of the rack is an important consideration when adding plenum or rack monitoring to an animal health monitoring program. In addition, this study demonstrated that depending on the ventilated rack design, rack testing can be a reliable method for detection of some, but not all agents found in contemporary mouse colonies.

PD46 Contemporary Prevalence of Infectious Agents of Mice and Rats in Europe and North America

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Laboratory rodents can serve as sources of infectious agents for disease outbreaks in laboratory animal facilities. Agents with a higher prevalence pose an increased risk of introduction into naive rodent populations. Knowledge of infectious agent prevalence can help with risk assessments to develop agent exclusion lists and determine testing frequencies for excluded agents.
In this study, the prevalence of infectious agents in samples submitted for diagnostic testing in the year 2014 is presented as the percentage of positive samples of the total samples tested. Tests to detect viral, bacterial and fungal pathogens as well as endoparasites and ectoparasites were performed on up to 88,000 rodent samples. The most frequently detected viral infections in mice were Murine astrovirus (75.79%), Murine norovirus (34.38%), Mouse parvovirus (0.46%), Mouse hepatitis virus (0.25%) and Mouse rotavirus (0.12%). The most common viral infections in rats were Rat theilovirus (2.86%) and Rat minute virus (0.27%) and Rat coronavirus (0.26%). Entamoeba muris was the most prevalent nonpath-

ogenic enteric protozoan detected in mice (19.20%) and rats (7.94%). Pinworms (2.41%) and fur mites (1.10%) were the most frequently detected pathogenic parasites in mice. In rats, pinworms (4.44%) were most prevalent and fur mites were detected infrequently (0.16%). Corynebacterium bovis (15.07%), Helicobacter spp. (12.96%) and Pasteurella pneumotropica (12.11%) were the most prevalent bacterial pathogens detected in mice. The prevalence of Helicobacter spp. (7.31%) and Pasteurella pneumotropica (1.96%) in rats was lower than in mice. Contemporary prevalence estimates of infectious agents in laboratory rodents can help shape decisions regarding agent exclusion lists, test selection and frequency for health monitoring.

PD47 Survival and Growth on different Zebrafish Strains fed with a combination of Dry and Saltwater Rotifer Diets

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In last few years, the number of zebrafish facilities using the saltwater rotifer, Brachionus plicatilis, as first feeding, has increased considerably. Zebrafish, like many other fish species in aquaculture can be considered a live food dependent fish, specifically during their first larval stages.
In this trial we analysed the effects of three different diets on survival and growth rates using larvae from three different wild-type strains and a mutant strain during pre-metamorphic and metamorphic stages (from 5 dpf- days post fertilization- until 14 dpf). The results showed that the quality of the larvae, in reference to survival and growth and raised using the control diet were the same for the willdtype strains (92-75%), but a slightly lower survival rate for the mutant was found (72%) but the mutant had a significantly lower growth rate. No significant differences were found between the control and diet groups in survival data for two of the wildtype strains at the end of the trial but we discovered significant differences on survival for one of the wildtype strains and the mutant strain between control and Diet B. This suggests that there could be other factors affecting larval survival that are independent of diet. Interestingly, after 14 dpf there were no significant differences in growth for the wildtype and the mutant strain that depended on diet. In contrast, control-fed larval fish were significantly longer than the diet A-fed fish in two of the wildtype strains. These results suggest that rotifers are suitable as a first fed for larval zebrafish but it is widely known that rotifers and artemia are deficient in some essential nutrients. Powdered dry food would facilitate in compensating for this imbalance. Further investigation is required into standardization of larval feeding protocols but these results suggest that further refinement depending on zebrafish strain is essential to achieve reliable results in scientific research.

PD48 The Prototype of post-operative care cage for sheep and swine; Usefull design for recovery

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Post-operative health care of sheep and swine after surgery has some difficulties for veterinarians and veterinary technicians. Animals be cared for after procedures to ensure their full recovery. After surgery during 24-48 hours, animals must be monitored and recorded well before taken home cage. The mission of the post-operative cage is to diagnose, treat, and prevent disease, pain, discomfort, or distress in sheep and swine. Cage designed for animal comfort during recovery time.
The post- operative charge covers required physical exams, routine monitoring and nursing care, extubation, routine antibiotics (e.g. ceftiofur or clavamox), furosemide (Lasix) if needed, medical record, and tech time to perform these procedures. Analgesics, re-intubation, fluids, wraps, IV catheterization, and any additional record-keeping (paper records, GLP records, etc) required separately. New post- operative cage designed for animal comfort during recovery time to help veterinarians and veterinary technicians. After surgery veterinarian and veterinary technician must be stand near of the animal for intensive care. Sometimes it takes long time and animals can be stressed easily cause of being far from home cage. Post- operative process should be management carefully to increase the survival rate after surgery. For all difficulties of recovery care process, cage designed for needs of animal and veterinarians. Post-operative cage has big area for different posture positions(1500x2200x2750 cm) and enough place for veterinarians to control animal in the cage (for ,catheterization, injection, drug treatments, physical exams,nursing care and etc.). Transporting cages can pass throught in to post operative cage door and can turn easily into the cage. Cage has adjustable heat panel inside the stainless steel walls and degree control panel in front of the cage. Top of cage there is a camera for following animal behaviors, pain, stress and feeding time. Also cage has a removable lifting belt on the roof to set on leg of animal when needed. Belts also help technicians to hold animal. Under the cage there is a stainless table with pipe to collect urine and to measure urine volume. Cage has noise panels and has wheels to move into the room or to move another room. Also it can be use as transporting cage so animal changes less cage while taken to the home cage. All parts of cages can removable and can be decontaminate by hydrogen peroxide. New recovery cage design more useful and comfortable for animals, so decrease the stress after surgery. Care unit veterinarians and veterinary technicians can record every steps of post-operative process smoothly and to do physical exams fabulously on sheep and swine.

PD49 First results on the growing influence of three different mouse feedings in a strict SPF barrier

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In our SPF mouse facility a slight outgrowth in the weight of the newborns in the C57Bl/6 J colony was realised. A reduced breeding outcome was recognised. Because of these findings, we started to try three different mouse feedings to find out which feeding regime is the best for our breeding mice.
Mice are animals with a high metabolic rate. Therefore, the right composition of their diet is of great importance. A lack of energy input in breeding mice immediately shows a reduced outcome in the number of newborns and in the growth capacity of the pups. In this study three different feedings were compared. Also, the impact of temperature regarding food consumption and body growth in mice was evaluated. The mice were housed at two different room temperatures as the environmental temperature has a major influence on the energy requirement in mice. (Karp CL, 2012) We observed six groups of mice with three different diets in two different temperature zones (22°C, 28°C). At least ten cages per group with two to five mice per cage, in total 300 C57Bl/6 J mice were used for this study. The feeding regime already started from the parent generation. The weight control started at the weaning and was continued once weekly for three months until the mice are expected to be fully grown. The diets

were provided by Ssniff (Ssniff, Soest, Germany) and were chosen by their energy density.1. M-Z Ereich is a diet with a high protein content and with increased energy density.2. VRF1 mouse and rat breeding is a diet with adjusted energy density for breeding matters.3. R-Z maintenance is a feeding with no special breeding adjustment regarding the energy density. All the diets were autoclaved before use and the mice were fed ad libidum.First results show a tendency towards faster growth in the high temperature room. The maintenance diet (3) also seems to have a negative influence on the growth of the pubs compared to the high-energy diets. During the run of the study the optimal composition of food and temperature regarding growth welfare and costs will be defined.

PD50 Refinement of the care and use of laboratory ferrets (Mustela putorius furo)

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Intravacc.

Ferrets are commonly used as an animal model, particularly in the study of human influenza viruses and Helicobacter associated gastritis. To optimize animal welfare and validity of these studies, refinement strategies should be implemented, such as provision of enrichment and timely and accurate recognition of pain. However, this information is currently lacking in ferrets, thereby warranting further research on these topics.
Materials and methods 1) We performed multiple consumer demand studies using a weighted push door in which we assessed the motivation and preference for different types and categories of enrichment (sleeping enrichment, social enrichment, foraging enrichment, water bowls, balls and tunnels) in seven female ferrets. 2) We investigated whether facial expressions, which are commonly used as an indicator of pain/discomfort in other laboratory animals, could be used as an indicator of pain in ferrets. For this purpose, we compared the facial expressions of 19 female ferrets at multiple time points before and after intraperitoneal implantation of telemetry probes. Two facial action units (FAUs) were also scored by blinded observers. Results 1) Ferrets showed the highest motivation for sleeping enrichment and were also highly motivated to gain access to social enrichment, water bowls and foraging toys. Within the different categories, ferrets were found to highly prefer a hammock (category sleeping enrichment) and a large water bowl (category water bowls), whereas no preference was observed within the category foraging enrichment. 2) Compared to baseline, ferrets showed various facial actions 5 hours after surgery: orbital tightening and position changes of whiskers, ears, nose and cheek. The changes in the first two FAUs could be reproduced by observers blinded for the time points at which the photographs were taken. Discussion and conclusion Results show that a hammock, conspecifics, large water bowl and foraging enrichment are preferred enrichment items for ferrets. Therefore, we recommend to offer these enrichment options in ferret housing. The changes in facial expression in the ferrets after surgery suggest that pain/discomfort can be recognised using FAUs in ferrets, which is a first step towards development of a ferret grimace scale. Results of these studies provide important building blocks for the refinement of studies using ferrets as a research model. Further validation studies using a control group will be continued.

PD51 Coping with animal utility and empathy in the lab

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Humans use other animals for a variety of purposes, whereby a strive for resource or time efficiency (utility) often clashes with understanding and consideration of the animals' needs (empathy). Among e.g. veterinarians and animal researchers the concept of moral stress has been studied - the gap between what you want to do and what you can/are allowed to do, or between what you ought to do (according to colleagues, policies), and don't want to do (according to own ethical considerations).
Material and method In order to better understand our often conflicted relationships with other animals, we will investigate the relationship between utility and empathy, both potential clashes and synergies. We will perform semi-structured interviews with laboratory animal technicians and researchers. Hence, this is a qualitative study leading to elaboration of informants' views and arguments. Results Preliminary results from a pilot study confirm the occurrence of moral stress, and show a perceived difficulty to combine utility (in relation to e.g. research, handling, costs) and empathy. Further results (from an ongoing study) will be used for an ethical discussion on the relation between these concepts. Discussion and conclusion It has been found that focus on utility and utilitarian ethics are correlated with lower emotional attachment and identification, e.g. important aspects of empathy. Further, people who use animals commercially, e.g. for food production or for research, often find it necessary to distance themselves from the animals in order to not get too emotionally involved or feel too much empathy with the animals. Hence there is a perceived clash between a general affinity to care for animals (empathy) and focus on their function in human use (utility). Different coping mechanisms to deal with moral stress have been found. L labelling of animals or using numeric names for them, as well as a lack of direct contact with the animals is common, lab technicians and animal caretakers sometimes single out an animal for a pet, and sometimes took home "rescues", and the term "sacrifice" is used rather than "killing". If there weren't a discrepancy between our often quite instinctive concern for the wellbeing of animals, and the way in which they are treated, it seems such coping strategies would not be needed. We are interested in both how informants reason with respect to such coping strategies, and in the ethical implications of moral stress for one's overall morality. The ethical elaboration will focus on autonomy, integrity and utility and the role of empathy and emotions in decision making. We envisage this mapping of clashes, synergies and strategies will contribute to formulation of better practice in handling moral stress.

PD52 Investigation of an In-Line Media to Simplify the Sampling of Exhaust Air Dust and Improve Rodent Pathogen Detection by PCR on an IVC Rack

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Bedding sentinels have been used for decades to monitor rodent colonies for pathogens, but continued investigations support that many pathogens are not easily detected in bedding sentinels. We have investigated exhaust air dust (EAD) PCR testing of IVC racks as an alternative to bedding sentinels which has initially relied on available sampling locations to provide a concentrated dust samples. Recently we investigated an in-line collection media to collect dust within the exhaust plenum.
An IVC rack without cage-level filtration was used to house study mice. To simulate a low (~5%) prevalence rate of infection on the rack, one 3-4 week and one 6-10 week pet shop mouse were placed together into each of four cages (8 mice total). At time zero, these mice were demonstrated to be shedding or infected with 20 rodent infectious agents. Soiled bedding was collected during bi-weekly cage changing of pet shop mice and diluted to 5% with soiled bedding from gnotobiotic mice free of opportunistic and primary rodent pathogens. Subsequently, bedding was also

transferred to 4 sentinel mouse cages containing CD-1 mice and 4 cages without mice. Sentinel mice were evaluated by traditional screening methods (serology, bacteriology, and parasitology) and PCR (fecal pellets, fur swabs and oral swabs) at 3 months. EAD samples collected by pooled plenum and hose swabs, sentinel cage filter and the in-line media were evaluated individually at 3 months by real-time fluorogenic PCR. All study cages were placed furthest from the EAD collection points. At 3 months post exposure, direct sentinel screening by PCR and traditional methods collectively detected 6 agents in at least 1 of the sentinel mice. Sentinel cage filter EAD testing detected 17 agents in at least 1 of the filters. Plenum and hose EAD (combined) testing detected 15 agents. The in-line media testing detected 19 agents. In addition to detecting more agents than bedding sentinel screening or other EAD samples, the copy number of target nucleic acid determined by real-time PCR was ten times or greater for most agents compared to other EAD samples. Additionally, the media sample also demonstrated higher nucleic acid copy numbers than the standard adhesive swab technique used to collect EAD from horizontal plenums.Bedding sentinel use for pathogen detection on IVC racks has been an unchallenged method due to lack of an alternative method for comparison. Our laboratory and others continue to identify the failure of bedding sentinels to detect relevant prevalent agents. This investigation and others have demonstrated the successful use of EAD PCR to improve rodent pathogen detection by detecting agents that are detected by sentinels and many more that do not. Last, but not least EAD PCR testing provides the means to reduce or eliminate the need for sentinel mice altogether.

PD53 Efficiency of soiled bedding transfer for transmission of mouse and rat infections to sentinels

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A systematic review (SR) was conducted to get insight into the effectiveness of transmission of infections to sentinels through soiled bedding transfer based on publications studying this subject in mice and rats housed in Individually Ventilated Cages (IVCs). This information is necessary to establish recommendations on improvements to the design of health monitoring programs using either a sentinel program, life animal sampling of colony animals or testing the IVC itself.
Fifteen original articles received from Pubmed, Embase, and Cab abstracts met the inclusion criteria of the. These papers showed that there is very little high-quality evidence to substantiate the efficacy of soiled bedding transfer. The design of the included studies differed substantially per pathogen with regard to dose of soiled bedding, exposure time, and sentinel strains used. Soiled bedding transfer only appeared to be effective for MHV, MPV, TMEV, Helicobacter spp., and fur mite infections. For other pathogens, such as MNV, EDIM, MVM, SDAV, Clostridium piliforme, and pinworms, too few data were available to be able to draw reliable conclusions. The published data cover only a part of all pathogens included in the FELASA 2014 guidelines. As most animal facilities consider these recommendations leading, additional studies are warranted to be able to draw final conclusions on the efficacy of soiled bedding transfer for the pathogens listed in these guidelines. It is important to complete the missing information and start with analysis of historical data and further soiled bedding transfer studies in the near future in order to confirm as soon as possible whether the use of sentinels is justified and whether sentinels should be incorporated into the design of health monitoring in IVCs or not.

PD54 Filobacterium rodentium, a new name of the cilia-associated respiratory bacillus

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The Gram-stain negative filamentous bacterium isolated from rat pneumonia, formerly known as the cilia-associated respiratory bacillus ('CAR bacillus'), induced chronic respiratory disease (CRD) in rodents. 'CAR bacillus' belonged to phylum Bacteroidetes but has sufficient identities to warrant a new family, genus and species, and has been recently named Filobacterium rodentium gen. nov., sp. nov. in Filobacteriaceae fam. nov., on the basis of biochemical, phenotypic, and 16S rRNA analysis [1].
[Materials and Methods] SMR-CT was cultured in conditioned medium (culture supernatants of Vero E6 cells cultivated by using Iscove's Modified Dulbecco's Medium supplemented with 10 % fetal bovine serum). All cell cultures and SMR-CT cultures were performed at 37 °C in 5% CO2 95% air humidified chamber. Morphological changes were monitored under phase contrast microscopy. Biochemical reactions were determined with the Rapid ID 32A anaerobic identification kit (bioMérieux). Fatty acid methyl esters were extracted and processed to specify fatty acid profiles. Previously obtained full genome data of SMR-CT were used to deduce 16S rRNA sequence and DNA G+C content. Phylogenetic analyses were carried out by using the neighbor-joining method and the maximum-likelihood method with Bootstrap resampling. [Results and Discussion] SMR-CT showed microaerobic, non-spore-forming, motile (gliding) without flagella, Gram-stain-negative, argentophilic, filamentous rods. The doubling time was 20–24 h. When cultured in ultralow attachment flasks, cells were 0.8–0.9 x 8.3–10.0 μm in size and presented singly in the planktonic state. Cells in conditioned medium on glassware grew in sessile state and made net-like structure on glass surface. The dominant cellular fatty acids were iso-C15:0 and anteiso-C15:0. The DNA G+C content was 47.7 mol%. SMR-CT and closely related strains of 'CAR bacillus' rodent-isolates formed a novel family-level clade in the phylum Bacteroidetes with high bootstrap support (98–100 %). From these results, a new scientific name, Filobacterium rodentium, was proposed for strain SMR-CT of 'CAR bacillus'. The type strain is SMR-CT (JCM 19453T, DSM 100392T). Several reports said that 'CAR bacillus' widely found in wild rodents. Now that rodent-type 'CAR bacillus' has a real name and SMR-CT is available from public collections, its character will be clarified soon.

PD55 Eradication of mouse parvovirus introduced in an SPF facility

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Mouse parvovirus (MPV) was imported into the experimental unit of an SPF mouse facility of about 5500 IVC cages by ordering mice from a commercial breeding company. A notification of MPV positive results was sent by the company on 14th of July. Mice originating from this company were traced: 20 cages with mice were introduced in the SPF facility. Serum samples were taken for ELISA analysis, cages were kept closed until the company confirmed their MPV positive status on 17th of July.

At that time, the 20 suspected cages were taken out of the barrier and the mice were sacrificed, with autoclaving of all material involved. On 29th of July, the results of the 20 cages turned out to be positive. MPV is a very resistant virus that is hard to eradicate. As the infected cages had been opened in the animal house labs for a month before we were notified about the infection, the complete barrier was considered contaminated and closed for scientists. A small 'parvo-team' (7 persons) was assigned for further care-taking, partial depopulation and disinfecting of the facility. This part of the facility consists of 3 rooms with 5 ventilated racks per room (140 cages per rack), with positive pressure differential relative to the corridor and 4 lab rooms. From each rack, samples from dirty sentinel mice and random serum samples were taken. Sampling of rack exhaust filter and dust in the lower plenum was performed. Later, only sentinels of the room and the dust swab of the plenum and filter of the rack in which the majority of the MPV positive imported cages were formerly housed, were found positive. The positive sentinels were killed and blood samples taken as positive control. New sentinel mice were brought in. Meanwhile, 70% of all cages (housing short term or relatively easy to repeat studies) were culled by the parvo team, to create free space. Changing stations were cleaned with Virkon S and gassed with hydrogen peroxide (H2O2), cages were changed in their original housing room while taking dry spot blood samples for ELISA and fresh faeces samples for PCR of every individual cage. Cages were kept closed until the results came in. The emptied ventilated racks were deconstructed, washed and autoclaved. One by one the housing rooms were emptied, cleaned thoroughly and filled with clean racks for H2O2 gassing. In the lab rooms, all disposables were discarded, all other material was mechanically rinsed and soaked in or sprayed with Virkon S solution. All labs were thoroughly cleaned and gassed afterwards. On 7th of September, all samples (both the faecal and serum samples) were found negative for parvovirus. Cages were changed, providing dirty bedding to the sentinels. Both the clean and dirty corridor, and the clothing area were cleaned and disinfected before scientists were allowed to enter the facility again. The first 3 months, sentinels were screened two weekly for MPV. Up to now, no positive samples were detected, and MPV is considered eradicated.

PD56 Challenges of the husbandry of wild house mice as model system for evolutionary and bahavioral studies

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House mice (Mus musculus) have spread from their origin in Southern Asia around the globe in several waves. They have differentiated in subspecies and have adapted to many habitats, including desert-like areas, arctic regions and oceanic islands. They have also a complex social system, including communal nesting, as well as a rich chemical and vocal communication. They are therefore an excellent model system to study the molecular basis of evolutionary adaptations and behavioral ecology.
We have obtained a range of populations, subspecies and species of wild mice from various regions of the world. The colonies were started with wild caught mice and stocks are maintained using a rotating breeding scheme to avoid inbreeding (HAN rotation). The successful husbandry of such mice requires several modifications compared to laboratory mouse husbandry. Wild mice have a much higher agility and are more easily stressed. For example, they are disturbed by noise from handling metal cages or loud noises, are influenced by the mood of caretakers, require a possibility for exercise and can vigorously defend their territory. We keep them in open cage systems with ample enrichment and running wheels. This allows them to get exercise, build nests for thermoregulation and cover and provides different hiding spots across the cage. Cage changes and setting up breeding pairs requires special tools and strategies. We run also a facility for experiments in semi-natural environments, where mice live and assort freely in a room for multiple generations. We routinely monitor for diseases and parasites. We do not have much problem with pathogens, but observed waves of infestation with mites, as well as ulcerative dermatitis as a consequence of bite wounds. Mice kept under high hygiene conditions appear to be particularly vulnerable to this, possibly because this puts them into a higher stress level. In this presentation we will discuss our solutions for a successful husbandry of wild mice, as well as show some examples of our work, including studies on ultrasonic vocalization and mate choice. The recording of ultrasonic vocalization under prolonged conditions and in different sex combinations has shown that females vocalize more than males, particularly in female-female encounters (von Merten et al. 2014). Mate choice studies have shown that mate preferences are at least partly determined through paternally provided information (Montero et al. 2013). The advantages but also the problems to keep wild mice in an experimental environment

PD57 The effect of long-term exposure to low electromagnetic fields (EMF) as an integral part of the housing system on anxiety-related behaviour, cognition and welfare in two strains of laboratory mouse

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Electromagnetic field (EMF) technology has the potential to improve scientific data capture and welfare assessment by allowing automated data collection (e.g. animal activity) from individual cages. However, it is important to determine any impact that a new technology itself may have on animal welfare, and previous studies have found contrasting results of EMF on laboratory rodent anxiety-like behaviour and cognition that may be due to different durations and/or intensity of the EMF studied.
Materials and Methods: We therefore investigated whether there was an effect of EMF experienced continuously over a six-week period, as an integral part of the animal housing system, on measures of mouse anxiety-related behaviour, cognition and welfare. We housed mice (N=80) of two strains (BALBc and C57BL/6J) separately in IVC cages (cage rack modified to accommodate EMF plates, with an intensity range of 5-100Hz) in groups of four, either with the EMF plate turned 'on' or 'off' (n=5). Some measures, e.g. behavioural observations and records of food and water utilisation, were collected at regular intervals, whereas measures of anxiety-like behaviour and cognitive performance were collected at the end of the study. Results: We found expected strong strain differences in most measures, e.g. latency to leave the starting square in an open field test: $F_{1,76}=6.9$, $P=0.0104$, with C57BL/6J mice moving away sooner, and interactions between strain and time for those measures recorded at more than one time point, e.g. bodyweight: $F_{6,96}=7.442$, P<0.001, reflecting significant weight gain over time for both strains, but with BALBc mice weighing more. However, we found no significant effects of treatment (EMF 'on'/'off') for any measures, e.g. bodyweight: $F_{1,16}=0.021$, $P=0.886$; latency to leave starting square in the open field test: $F_{1,76}=1.3$, $P=0.254$; novel-object recognition test: $t38=1.586$, $P=0.121$.Discussion & Conclusions: These results indicate that, at least for the measures recorded here, there was no measurable impact on the behaviour and welfare of EMF exposure experienced continuously over a six-week period. Housing systems that include EMF monitoring technology may therefore be suitable for use without influencing either animal welfare or scientific outcomes.

PD58 Impact of naturally-occurring infections on biomedical research using zebrafish

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Biomedical research should be conducted using methods that produce valid, reproducible results in order to satisfy ethical and fiscal responsibilities. 1 Both clinical and subclinical naturally occurring infections can introduce variability that can lead to invalid or misinterpreted experiments in animal models, including zebrafish. 2 Therefore, exclusion of infectious agents from zebrafish colonies is a critical component of reducing confounding variability in biomedical research.
Several naturally-occurring infectious diseases in zebrafish colonies have the potential to cause high mortality epizootics, clinical disease, and/or elevation of colony mortality over time. Morbidity and mortality in zebrafish colonies can result in increased animal usage, repetition of failed experiments, loss of a valuable zebrafish model, or loss of balanced experimental designs. Even in the absence of clinical disease, subclinical infections can result in altered gene expression, altered cytokine levels, reduced growth, reduced fecundity, unexplained variability in experimental data, misinterpreted data (Type I and Type II errors), increased animal numbers to demonstrate statistical significance, and inability to replicate experimental results. Zebrafish embryos and larvae are also susceptible to infection, and mount an innate immune response. Importantly, cytokines have key roles in development as well as immunity. The most prevalent pathogen, P. neurophilia, is vertically transmitted, and subclinical infection with P. neurophilia has been associated with an altered neurobehavioral phenotype, as well as reduced growth and fecundity in stressed fish. Other prevalent zebrafish pathogens are Mycobacterium spp., and Pseudocapillaria tomentosa. All mycobacterial species that infect zebrafish cause chronic granulomatous inflammation which is problematic for nearly every type of research that uses adult fish. Moreover, Mycobacterium haemophilum and M. marinum often cause clinical disease and mortality, and M. marinum has been shown to act as a tumor promoter in other fish species. Similarly, natural infection with P. tomentosa altered the results of a carcinogenicity study when P. tomentosa-infected zebrafish developed significantly more tumors than uninfected zebrafish in the same treatment group.3 Successful exclusion of zebrafish pathogens from research colonies will be facilitated by the use of purpose-bred specific pathogen-free animals, approved vendor lists, pathogen exclusion lists, quarantine practices, disinfection, routine sentinel health monitoring, and environmental monitoring.

PD59 Cellulose pellets bedding: Animal Welfare and Efficiency together

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In order to improve animal welfare and decrease costs, a study was carried out at the same conditions on bedding for rodents. A new one based on cellulose (pellets), coming from a different commercial company was used.
One group of one hundred of Individual Ventilated Cages for mice was used. Ten of them were used as controls with the highest occupancy permitted by law (Directive 2010/63/EU) and the rest with the real number of animals housed during experimental procedures. All cages were daily observed at least during two weeks and control of bedding was also checked periodically by physical, chemical and bacteriological methods (e.g. ammonia levels, images, bacteriological cultures). As the wooden materials did not reach the required results, a new organic –cellulose- was incorporated to the study. It was found that the cellulose pellets bedding complied with the following:

1. Enlarging the bedding viability: A minimum of fourteen days was reached with no cage changes.
2. Decreasing animal stress letting them to stay more time in its environment with no changes.
3. Bacteriostatic ability that limits the growth of microorganisms.
4. High capacity of absorption / desorption balance.
5. Faeces are displaced to the bottom of the cage beneath the bedding.
6. Low levels of ammonia.
7. Reduction of costs in cage's maintenance.
8. Reduction of air renovations in IVCs.
9. Improving environmental sustainability.
10. More time availability for the technicians and caretakers in order to carry out the legally required daily follow-up of animal welfare.

PD60 Construction and development of animal facilities - an integrated approach

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Construction and development of animal facilities are often complex and enormous financial projects. An early integration and coordination of all expertise and knowledge is crucial for the optimal performance infrastructure, ensuring strict compliance with guidelines, legislation and suitable security at all levels. This presentation aims to highlight the advantages from an integrated skilled approach.
This work illustrates the systematization of infrastructural and operational requirements of a vivarium for the subsequent definition of the most appropriate solution. The approach for construction and development of integrated animal facilities solutions is first defined by the space purpose (immediate and future) and underlying constraints. These are weighted in all stages of the process [1-diagnosis; 2-conception; 3-engineering; 4-execution; 5-training; 6-maintenance] and are materialized in a solution where all elements (spatial organization, infrastructure, furniture and equipment) are harmonized for maximum procedural efficiency, fulfilling both regulation and user requirements. This approach begins with user requirements identification (animal species/quantities, investigation purpose, etc.) followed by definition of workflows, spaces interconnections and technical requirements in terms of infrastructures and environmental control (light intensity/color, rooms temperature/humidity, air changes/filtration, etc.). All this information is weighted in a matrix and analyzed by a multidisciplinary team of qualified consultants, engineers and architects in order to match each specialty individually and the project as a whole ensuring that the assembly runs smoothly. This presentation gives some practical example and shows that in addition to strict compliance with the guidelines and technical rules, it is essential a strategic global thinking applied to all vectors in order to achieve maximum potential of the vivarium. Results from this approach showed consistent reduction in costs and times to achieve optimal functional requirements.

PD62 A review of alternatives to problematic wire grid floors in large rabbit husbandries and the need for regulations in Directive 2010/63/EU

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Rabbits for agricultural use as well as for research purposes are bred in very large husbandries using wire grid as floor system. Several studies investigated effects of floor type on behaviour, health and performance, mainly of farmed rabbits. Due to serious criticism of the wire grid system, that causes severe foot problems, most studies compare wire grid to plastic or straw-bedded floors. This review gives an overview of the current state of research on floor designs in rabbit husbandry.
Straw bedding is discussed as a welfare-friendly alternative. It can improve physical comfort and stimulate and allow exploratory and foraging behaviors. However, in terms of hygiene, labor and costs straw bedding is regarded critically. Moreover, in fatting rabbits lower weight gains were achieved on straw-bedding (1). Plastic floors seem to be a useful alternative. Studies show that plastic floors are preferred by rabbits (2) and that they are at least equal if not better than wire grid floors regarding welfare and health (3). There are hardly any studies on effects of floor types for rabbits bred for research purposes, but it can be assumed that the needs of the rabbits are the same, regardless if they are kept for research or agricultural purposes. Although there are animal protection regulations regarding floor design for rabbits in several EU-Countries, the EU-Directive 2010/63/EU lacks any regulation for floor design in rabbit husbandries. Laboratory Animal Science should not only investigate husbandry systems on their impact on the animals, it should also use the gained knowledge to improve and change existing systems. For instance, in Austria, wire net floors are forbidden by the THV 1 (order to animal protection law) since 2010. Circular orifices commonly used for laboratory purposes must be maximally 12 mm in diameter for pups (until about 4 month) to suit the THV 1. However, such a floor is not commercially available. A prototype of a floor with circular orifices suiting both the regulations for laboratory as well as farmed rabbits was developed and is being tested at the moment. Animal protection laws all over Europe, as well as the EU-Directive 2010/63/EU should be standardized and adjusted according to current scientific knowledge to improve rabbit welfare and health in Europe. Moreover, producing rabbits for scientific and agricultural purposes in one and the same husbandry system could be an interesting business model and would be in compliance with one of the 3R's, Reduction. At the moment, due to the different regulations, this is hardly possible.

PE1 Accreditation of animal facilities for the production of laboratory animals. Production License

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The National Center for Laboratory Animal Breeding (CENPALAB) produces different species and experimental animal lines, that the country needs, under the regime of good practices, contributing to the development of the laboratory animal science and technology in Cuba. In this work, the Quality Management System (QMS) and Good Practices (GP) applied in the production of laboratory animals in CENPALAB for obtaining the accreditation and licensing of these productions in the country is exposed
Materials and methods: An assessment of the quality system implemented was made through the analysis of its performance indicators and the interaction of this with GP. There have been inspections and audits by centers and national and international regulatory bodies to assess the performance and effectiveness of the quality system, production system, among other indicators. Results: With the implementation of the quality system and good practices in the production of experimental animals, it is guaranteed the production of these biomodels with a microbiology and genetic quality. Inspections and audits received by the centers and regulatory bodies demonstrated the reliability of these productions and recognized the effectiveness of the functionality of its facilities, training of human resources and the quality of obtained scientific productive results. With these external audit processes, it was achieved to demonstrate results that have contributed to granting for more than 10 years the license for possession and reproduction of animals for experimentation by the Institute of Veterinary Medicine of the country, as well as other accreditations by other regulatory institutions. Discussion and Conclusions: The implementation of the QMS and implementation of GP in obtaining and breeding experimental animals has allowed meeting specifications, legal standards and customer expectations, ensuring the quality and safety of the final product. With this production license and accreditations, production processes ensure a defined and controlled quality, enabling the successful use of these biomodels, which are very necessary for the development of the Cuban biotechnological and medical pharmaceutical industry.

PE2 The Interspecies Database: reducing the number of laboratory animals by choosing the relevant experimental animal model

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According to the European Directive 2010/63, researchers are obliged to consider the 3Rs (replacement, reduction and refinement) when designing and performing procedures involving animals. To accomplish this, the latest information about 3Rs-methods has to be identified. Databases and informative websites can facilitate retrieval of specific 3Rs-related information for scientists, but also for the institutional Animal Welfare Bodies, project evaluators (DEC's) and the Competent Authorities.
These databases and websites save time-consuming searches, facilitate completeness and contribute to the 3Rs. To be successful, they should be easily found, accessed, managed and updated. In addition, relevant data should be easily retrieved. By its 3Rs database programme, the 3Rs-Centre Utrecht Life Sciences (ULS) facilitates the search for and implementation of 3Rs methods. One of the databases within this programme is the Interspecies Database (www.interspeciesinfo. Com). This database provides insight into physiological, anatomical and biochemical parameters of different animal species and humans. By using the database, researchers can make a smarter design of animal experiments in terms of choice of a relevant animal model. This contributes to research quality and could result in a reduction of the number of experimental animals. The Interspecies Database is consulted by users worldwide. In the coming years the database will be extended with information on and data from more animal species, organs and parameters. The database was originally established by the National Institute for Public Health and the Environment (RIVM, the Netherlands), which will remain responsible for the supply and quality of new data.

PE3 Unique e-learning module on systematic reviews and meta-analysis of animal studies; an interactive introduction to the basic principles and power of this methodology

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A systematic review is a method to obtain a reliable, structured and transparent overview of all available studies on a specific (research) question. Within evidence-based medicine systematic reviews of clinical trials are common practice. In animal research, the systematic review methodology was hardly known. Only 7 years ago, the method was introduced in the field and implemented (e.g. in educational programs) (Leenaars et al. 2012a).
After careful steps in the beginning, the methodology becomes more and more recognized to be a valuable tool to objectively evaluate the value of animal studies and to bring transparency in the possibilities of translating animal data to humans (De Vries et al., 2014). SYRCLE has developed several tools and educational programs to facilitate and ease the process of performing a systematic review of animal studies (Leenaars et al., 2012b). The Dutch government accepted two motions of the House of Parliament concerning this topic. One motion was: "systematic reviews should become the norm within laboratory animal science like it is within evidence-based medicine for humans". The second motion requested "education on systematic reviews of animal studies in the Course on Laboratory Animal Science". As a result education on systematic reviews of animal studies is an obligatory part of the education of new animal researchers in the Netherlands since September 2015. SYRCLE was contracted by the Ministry of Economic Affairs to facilitate this education. In order to make this education available in a place and time independent manner, an e-learning module in English was developed. The module includes an introductory video scribe and several interactive assignments for all important steps of a systematic review of animal studies. After completing the e-learning module, the participant is aware of the potential of the methodology, its basic principles and its most important steps. As a follow-up, participants can enroll in one of the practical hands-on training programs in systematic reviews and meta-analysis of animal studies (www.syrcle.nl). The first e-learning module on systematic reviews of animal studies was officially launched by Susanna Louhimies (EU commissioner) during the international conference 'Laboratory Animal Science 2.0' which was held on 15 October 2015 in Nijmegen, the Netherlands. The poster will include screen shots of the e-learning as well as experience of participants with the e-learning so far. Get an interactive introduction into systematic reviews of animal studies by following the free e-learning here: https://syrcle.ekphost.nl (registration code: syrcle).

PE4 Evidence-Based Research Network (EBRNetwork) – A call to action for evidence-based research

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On behalf of EBRNetwork Steering Committee: Karen A. Robinson, Klara Brunnhuber, Robin Christensen, Peg Ford, Maureen Dobbins, Bertil F. Dorch, Marlies Leenaars, Hans Lund, Malcolm Macleod, Mona Nasser, Hanna Nykvist, Matt Westmore.

Background Efficient use of earlier research provides a powerful rationale for starting a study and a context in which to set the study results. Explicit use of earlier research, through the conduct of a systematic review, is also necessary for the design of an efficient and informative study. Yet research shows that there is inadequate and biased consideration of earlier research. MethodsIn Bergen, Norway in December 2014, the Evidence-Based Research Network (EBRNetwork) was initiated to promote the efficient and explicit use of existing research when new research is planned. Results We will present the aims, structure and activities of the EBRNetwork. Current activities include using peer-reviewed publications and social media to better inform researchers, funders, editors and the public. We will present for consideration the 'Bergen Statement on Evidence-Based Research'. Conclusion The new EBRNetwork is an international collaboration that aims to ensure that no new studies are approved, funded or published without systematic review of existing evidence; and works towards more efficient production, updating and dissemination of systematic reviews. The Network issues a call for participation. Affiliations of steering committee members:Karen A. Robinson, Johns Hopkins University School of Medicine; Klara Brunnhuber, Clinical Evidence and Best Practice at BMJ Publishing Group; Robin Christensen, Odense University Hospital, the Parker Institute, University of Southern Denmark; Maureen Dobbins, School of Nursing at McMaster University; Bertil F. Dorch, University of Southern Denmark Peg Ford, Ovarian Cancer Alliance of San Diego; Marlies Leenaars, SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE), Radboud university medical center; Hans Lund, University of Southern Denmark & Bergen University College; Malcolm Macleod, Centre for Clinical Brain Sciences at the University of Edinburgh; Mona Nasser, Plymouth University Peninsula School of Medicine and Dentistry; Hanna Nykvist, Bergen University College; Matthew Westmore, NIHR Evaluation, Trials and Studies Coordinating Centre, University of Southampton.

PE5 Changes in attitudes towards animals after following laboratory animal science education

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Scientists need to follow suitable education before being allowed to set up and perform animal experiments (European Directive 2010/63/EU). Our department offers a certified course on Laboratory Animal Science (LAS) meeting these education requirements for biomedical scientists. Next to providing relevant LAS knowledge, the most important aim of the LAS course is to build on their attitudes towards animal use and the animals (emotional) demands. In this way we aim to contribute to animal welfare.
To establish if this goal is reached and if it is a specific effect of following a LAS course or a more general biomedical science course we conducted an anonymous survey. This survey was performed at the beginning and at the end of both our LAS courses and a general biomedical course, changes in attitude scores of both courses were compared. Materials and Methods: Students enrolled in the LAS course are mainly researchers at the onset of their animal research career in the Netherlands. We took the survey before and after the course (the course follows FELASA C guidelines) with questions about different categories of animals; pet, pest, profit (PPP scale; Taylor et al., 2009) and laboratory. In addition, the same survey was performed on students before and after another biomedical course at the department of pharmaceutical sciences. For the laboratory animals category, we distilled 9 questions from a list of 36 questions in a validation study to differentiate the most discriminating questions on attitudes towards laboratory animals. Educational background, nationality, age, gender and upbringing were taken as demographic measures and were accounted for in the statistical analyses. Results/Discussion: Regarding the effects of the LAS course on student attitudes we have several hypotheses. We hypothesize that "the PPP scale is useful to investigate possible attitude change in the LAS course", that "Attitudes of students change more after following the LAS course than a more general biomedical course" and that "Differences in attitudes are influenced by the gender, upbringing and educational background". Results will help us to get more insight into our education and reflection on that will help to further improve our education.

PE6 Harmonization of methods for studying the toxicity of drugs according to 3RS in Russia.

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Beginning in 2012, Russia's active work on harmonization of domestic and foreign methods of preclinical drug safety. The purpose of preclinical drug safety is to obtain complete and accurate information for the safe conduct of clinical trials. Therefore, the scope of innovative safety studies of the drug should include study of general and specific toxicity and can be added depending on the kind of innovative drug, its potential mechanism of action, the chemical structure and other factors.
There is a fairly large list of methods for study toxicity, both in Russia and in other countries. Depending on the kind of innovative drug, the dosage form, route of application, possible toxicity data must be selected methods to obtain the full range of data safety. For example a method for studying acute toxicity, as described in the Guidance on pre-clinical research of medicines edited Mironov (2012) suggests research in a wide dose range with the start of all groups at the same time. This method may be preferred in the case of availability of information on the expected LD50. If such information is not available, then we run the risk of not "get" in the dose and have to choose other methods. For this case, a good approach proposed at the OECD protocol number 425, 420 and 423. They suggest pilot studies, as well as the sequential administration of a substance to each animal at different doses. It is reduced the number of animals in research and enter the desired LD50. It is also important to use different methods to assess the chronic toxicity according to the OECD and the Russian recommendations. The choice of method should be driven by the data on the drug, as well as the humane principles of using animals. To comply with the principles of 3R, optimization studies and the harmonization of methods of toxicity studies must perform the following tasks:1. Effective implementation of the principles of 3R "Replacement, reduction" is achieved by executing the following tasks:· Detailed analysis of the available data on the toxicity of the drug and its analogs;· Selection of the

most appropriate methods of research;· «in vitro» screening;· Consider replacing animal studies on research methods «in vitro»;· Justification of the choice of doses and dose step;· Prediction of the possible results and the choice of the method of analysis data;· Selection of the species and sex of the animals, the use of a minimum number of animals.2. Principle "Refinement" is achieved by executing the following tasks: · High professionalism of the staff providing care for animals and researchers; · High level of methods the collection of material from animal and other experimental methods; · The use of modern methods of anesthesia and analgesia; · And others. Thus, harmonization of methods for studying the toxicity of drugs according to 3RS necessary to achieve a high degree of reliability, efficiency and humanity of pre-clinical drug safety in Russia.

PE7 Progress with the Education and Training Platform for Laboratory Animal Science (ETPLAS) initiative

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Education and training is an important contributor to the 3Rs and is aimed at increasing the quality of both animal welfare and science. The Education and Training Platform for Laboratory Animal Science (ETPLAS) provides a portal for exchanging information on laboratory animal science education and training with all persons involved in this work.
It is mandatory that staff carrying out procedures, designing procedures and projects, taking care of animals or killing animals should have received instruction in a scientific discipline relevant to the work being undertaken and have species-specific knowledge. In this context, the initial focus of ETPLAS is to build a database of training providers with a point of contact for trainees across the EU who can offer courses meeting the requirements of Directive 2010/63/EU.ETPLAS has been established to enable information sharing and communication between EU Member States' authorities, accrediting/approval bodies, training providers and trainees involved with all aspects of education and training (E&T) in laboratory animal science (LAS). The Steering committee of ETPLAS is composed of representatives from all these groups and has developed a database of Contacts to aid communication. A Reference Group of contacts provides additional review and input to the work of the Steering Committee. ETPLAS has Observer status at the meeting of National Contact Points of the EU competent authorities where its work is shared and discussed. The aims of ETPLAS are to provide:· a forum for exchanging information on LAS education and training for all stakeholders· training providers with the necessary information to establish additional education and training courses· the user community with information on available education and training courses including continuing education (CPD)· authorities/employers with the necessary information to facilitate the process of mutual recognition of education & training to promote free movement of personnel involved in LASETPLAS has developed a website www.etplas.eu which is intended to be a one-stop shop for all involved in LAS. In addition to information on available E&T courses, there is the intention to provide links to courses and material suitable for CPD.

PE8 The CECAD- in vivo Research Facility Presentation of the Animal Facility of the Cologne Excellence Cluster

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CECAD's in vivo Research Facility (ivRF) is an animal facility with state-of-the-art technology, designed and run in line with the latest scientific findings, legislation, ergonomics, and especially animal welfare.
The CECAD (Cluster of Excellence - Cellular Stress Responses in Aging-Associated Diseases) research center includes a state of the art mouse facility (CECAD in vivo Research Facility, ivRF) with a capacity of more than 20,000 IVC cages. The ivRF comprises five functionally different barriers with distinct hygienic levels. Each barrier is operated independently. Centre piece of the animal facility is the fully automated cage processing area providing supply of the barriers. A comprehensive laboratory for transgenic services is directly affiliated to the CECAD ivRF. Transgenic services comprise the production of genetically modified mice, hygienic embryo transfer, cryopreservation of sperm and embryos as well as in vitro fertilization. The CECAD is promoted by the Excellence Initiative of the federal and state governments (DFG / WR). Planning, design and implementing of the animal facility as well as conceptual incorporation of the ivRF in the CECAD will be presented.

PE9 The Faceless Mentor: The Pros and Cons of On-line Training

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Reduction in face-to-face courses being offered by Australian colleges has resulted in fewer qualified animal technicians entering the research industry. To counteract the deficit, a number of establishments are offering fully online training at all levels in areas of animal care and technology. A positive argument for e-learning in some industries has been well established, but does this relate to the challenges faced when teaching and learning practical subjects?
By means of a survey carried out in a research institute and a college situation, this study examines the e-learning and F2F experiences of both trainers and trainees and explores the advantages, disadvantages and relevance of both systems in the research animal setting. An anonymous questionnaire was prepared. The first questionnaire was completed by trainers from both educational establishments and research institutes. The second questionnaire was completed by a trainee cohort, which included graduated Diploma and Certificate III Animal Technology students and research institute animal care staff, thus representing different qualification levels. All participants had experience of both online and F2F education. All questionnaires were identifiable solely by cohort and all included identical questions on perception, attitude and satisfaction. Results were collated and analysed. Whilst both systems achieved an adequate level of satisfaction with outcomes of the learning process being

met, both systems had advantages and disadvantages in terms of technical requirements, communication and time required for completion. Obstacles associated with the e-learning process were defined and parameters suggested for improving future e-learning experiences, resultant competencies and ultimately animal welfare.

PE10 The analysis of inspection reports in animal facility at Seoul National University Hospital

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Department of Experimental Animal Research (DEAR) at Seoul National University Hospital (SNUH) obtained GLP (Good laboratory practice) certification from MFDS (Ministry of Food And Drug Safety) in Korea in [2003], and was fully accredited by AAALAC in [2007]. Since then, we have performed several inspections in accordance with GLP, IACUC (Institutional Animal Care and Use Committee), and AAALAC.

According to the results of 10 times of GLP-QA (quality assessment), 9 IACUC, and 2 AAALAC inspections for last 5 years, total number of items noted as needing immediate remedial action was 732, 226, and 25, respectively. We classified total 983 items according to 6 categories, which were animal care and use, equipment, environment, personnel safety, facility, and drug. Indicated items about animal care and use were the highest frequency (277 of 983, 28.2%) among the categories, followed by drug (161, 16.4%), facility (157, 16.0%), equipment (156, 15.9%), environment (152, 15.5%), and personnel safety (80, 8.1%). A comparison between 2011 and 2015 reports indicated that environmental and equipment aspects were decreased from 33.3% to 9.0% and 27.8% to 7.8% respectively. But items about facility, animal care and use, drug were increased from 6.3% to 27.1%, 19.2% to 28.9%, and 9.4% to 18.7%. Also, personnel safety was slightly increased from 3.9% to 8.4%. Environmental and equipment aspects were found to be improved by regular inspection and post-evaluation whereas items about facilities were increased possibly due to relatively decrepit facility. Also, recent social trends and guidelines associated with animal welfare seem to affect the increased animal care and drug items. In conclusion, we suggest that regular inspections and post-evaluation programs are necessary not only management and improvement of animal facility but also continuous update of standard operating procedures. Therefore, animal institutes should retain the standardized staff educational and assessment program.

PE11 Our experience as LAS course providers

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The concern of public opinion on animal experiments, the 3R's adoption and the demand for compliance to Directive 2010/63/EU prioritizes education1 in LAS in order to conduct experiments humanely, scientifically and ethically. Therefore, since 2012, we have established an annual institutional LAS educational program2 and recently introduced a 5-days international course based on modules-structure3 that was assessed and rated with 27 credits of CME – CPD according to international criteria.
Our courses special objectives were i) the presentation of a content adapted to the needs of the trainees, ii) the integration of different LAS disciplines within a course, iii) the acquisition of competence via practice and iv) the assessment of course utility 7-months after its completion, in order to gain realistic feedback on its pedagogical value and quality. Therefore we established 3 types of course evaluation surveys, using a pre-course (Q1), a during-course (Q2) and a post-course (Q3) anonymous questionnaire using free-access google forms and hardcopy formats addressed to trainees. Q1-electronic survey tried to extract information on the target group of the trainees, their background and their course expectations. Q2-hardcopy was a detailed in situ course quality evaluation. Q3-electronic survey was addressed to international course participants' 7-months after the course end. The total number of trainees was 159 persons distributed in 4 courses. The course content was adapted to A/B/C functions and was consisted of both theory and practice. All courses reached mean score of 4.6 out of 5 (ranging 1-5, 5=excellent). Q1 revealed that 50% of the participants were PhD students, 36% were biologists and 73% had already LAS experience. Q2, among others, revealed that main course weakness was the exceeded speech time, while main strengths were the clear content, the helpfulness of the trainers, the quality of practical sessions and the participant's interactions with the trainers, while 10% asked for more advanced techniques. In Q3 survey, where 23% of the trainees participated, all gave high rate to the overall course quality, 45% used more than 5 times the practical handbook, 50% started to monitor endpoints, 50% introduced safety procedures in their routine and all of them introduced the 3R's concept to their colleagues. Our data as LAS course providers suggest that our target group is PhD students with LAS experience, but with limited knowledge of the 3R's concept. Trainees declared that important benefits were the endpoints and severity degree monitoring, the animals' gentle handling and practicing of common techniques. They appreciated the availability of trainers during the course and didn't contact anyone afterwards. We suggest the use of a quality course survey after a significant time of period, as an extra tool for assessing quality of LAS courses.

PE12 LAS education and training in Portugal – the contribution of SPCAL

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The Portuguese Society for Laboratory Animals Science (SPCAL) has been organizing and promoting laboratory animal science (LAS) education and training courses, promoting responsible research and the 3Rs in Portugal. SPCAL pioneered the implementation of a modular strategy of training, to provide researchers easier access to specific education in LAS, which has had a high impact on the number of researchers qualified and accredited for the use of animals for scientific purposes in Portugal.
As a scientific non-profit association focused on the improvement of animal welfare and scientifically sound use of animals in science, SPCAL began organizing education and training modular training activities in 2005, and include both practical courses – directed to researchers and technicians – and theoretical courses, mainly directed to researchers. The courses were designed according to FELASA recommendations and there has been a continuous dynamic effort from SPCAL to adapt the courses to the recent European Commission recommendations, following the implementation of Directive 2010/63/EU. The courses cover the training of persons to perform functions A, B, and D, according to this Directive.SPCAL

practical courses in LAS have been held in several institutions in Portugal, not only in the capital, but increasingly decentralized to the North and South of the country. SPCAL has also organized or supported other LAS-related education and training initiatives, such as workshops on experimental design and statistics, microsurgery, public communication of animal research, or training of animal welfare bodies.SPCAL has also been an active and constructive voice in the debate concerning the use of animals in science, in Portugal, while promoting the ethical use of animals, best practice and transparency in animal research in Portugal. In conclusion, SPCAL education and training courses have given a major contribution for the qualification of technicians and researchers, needed to obtain mandatory accreditation by the Portuguese Competent Authority (Direcção Geral de Alimentação e Veterinária - DGAV), as well as for the dissemination of the 3Rs principles and a responsible research in Portugal.

PE13 Innovations in the design and equipment of a multifunctional training area for medical and research staff for major, minimally invasive and interventional surgery.

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Animal models have relevant role for IMR learning, assuring no risk for patients. Surgical techniques require a period of specific learning, based on the precision and its growing complexity. Scientists who perform scientific procedures, must meet the refinement principle (D EU 63/2010), having to receive specialized training about 3Rs. The CTF is a unique design with specialized technology adapted to train IMR and research staff, optimizing the refinement principle in laboratory animals.
Objective Design an area with the latest techniques according to the principle Refinement in animal models, while achieving excellence in surgical and in interventional radiosurgery techniques. Material and methods Multifunctional area. This versatile area has 8 workstations and allows the development of various educational outreach and scientific innovation. Each station is equipped with a transportable stretcher with hydraulic system. A monopolar and bipolar electrocautery, surgical lamp with built-in digital display and with articulated and rotational arms. Digital presentations can be projected. The tower O2 and air connection/extraction provides hard disk with USB ports and direct connection to digital cable screen. Monitoring for basic and advanced vital signs are incorporated to 8 latest technology ventilators. 6 stations have mobile panels where station can be individualized. In the room, there are 3 digital cameras, 2 auxiliary large monitors and a digital presentation screen for a digital projector connected to hard disk of the central station. Oratory's teacher with portable microphone is controlled by an image and sound cabin controls. There are also 2 digital video-laparoscopy equipmentArea of interventional techniquesThis area consists of one main and one auxiliary rooms. The main provides 3 digital radiosurgery systems in portable C arc generator, camera incorporated into a 2 surgical lamps, a plumbed room with 2 plumbed stretchers and a digital ultrasound scanner with different probes (12, 7.5, 5.5 MHz). Results The activity of CTF has led since its inception to the celebration of 470 medical specialization courses for 9556 students (national and international), of which 50% have been hospital residents. Also, learning programs for 28 residents of various medical specialties have been enabled. Discussion and conclusions: Design of these areas differs from other existing centers in their adaptive capacity to the workshops structure: a) rotating groups for different sessions simultaneously; b) same simultaneous practice for all groups, tutored at each station or by a main teacher from the central station. Also, direct broadcast sessions for all over the world have been possible. These investment in high technology has helped to maintain anesthetic and analgesic status in animals supervised everytime by CTF technicians and veterinarians. Consequently, the Refinement principle has been carried out effectively from a very accurate vital signs monitoring.

PE14 VETCEE: Veterinary Continuous Education in Europe Competences for the VETCEE accredited programmes in Laboratory Animal Science and Medicine.

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Veterinary Continuous Education in Europe (VETCEE).
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[3]European College of Laboratory Animal Medicine (ECLAM).

Continuing Professional Development (CPD) is crucial for all veterinarians to develop and maintain the required expertise. VETCEE is a joint initiative of the veterinary profession. In collaboration with a European network of experts, it runs an accreditation scheme for structured CPD for practicing veterinarians. VETCEE ensures that the level/quality of veterinary CPD programmes in the different countries meets the Standard and facilitates their mutual recognition across in Europe.
VETCEE was established in 2014. It has developed a general standard for structured CPD for veterinarians complemented by Dossier of Competences (DoC) for the different animal species. In collaboration with European associations with expertise in the different species has adopted so far five DoC, namely in Companion Animal Medicine, Porcine Health Management, Equine Medicine, Bovine Health and Production and Laboratory Animal Science and Medicine.VETCEE Lab Animal Science and Medicine dossier was developed in collaboration with the European College of Laboratory Animal Medicine (ECLAM), the European Society of Laboratory Animal Veterinarians (ESLAV) and European Veterinarians in Education, Research and Industry (EVERI). The experts of the three organizations considered the principles of the VETCEE Standard as well as of the EU Education and Training Framework and Directive 2010/63, when drafting their proposal. The VETCEE accredited programmes should consist of approximately 750 hours of blended learning (equivalent to 30 ECTS) over a period of approximately 3 years. Candidates must hold a veterinary degree, be qualified for at least one year and have worked for a time equivalent to one year in laboratory animal medicine before enrollment to the programme; Programs should be structured in modules and accreditation is awarded to entire programmes. Programme providers should have a policy and associated procedures for the assurance of the quality and standards of their programmes and awards. Training and assessment of candidate veterinarians can be delivered in the native language, but applications for the VETCEE evaluation and supporting documentation must be in English.VETCEE only accredits programmes according to the Standard and does not award qualifications to individual candidates. Programmes have to be revaluated every five years. The evaluation of a programme is conducted by a panel of individual experts appointed by VETCEE Board. The panel of experts consist of at least one Chair, one academic and one practitioner with experience in laboratory

animal medicine. All experts subject to a no-conflict and confidentiality agreement. The panel provides a written report with recommendations to the VETCEE Board, who makes the final decision. The programme that passes successfully the evaluation is granted the VETCEE approval logo. VETCEE Standard and DoC are under regular update in order to meet with the developments in veterinary medicine.

PE15 The welfare of the transport.

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Janvier Labs.

The purpose of this study is the analysis of the real effect of the transport on rodents.
2 rodent strains have been used to carry out this study. A very calm strain, the outbred rat strain Wistar, RjHan: WI and a very nervous strains, the inbred mouse strain C57BL/6JRj, the most used inbred mouse strain for animal experimentation. The study concerns 2 types of transport: short travel (300 km) vs long travel (1,000 km). To evaluate the stress level during transportation, the weight of the rodents, the quantity of food and water consumed and the external aspect of the animals were used as parameters. The transport conditions of the rodents for this study have been the same as these for all rodents delivered by our transport department at JANVIER LABS. The results of this study show that transport effect is very variable according to the species, the strain and the transport duration. The shorter the travel is, the more the rodents are stressed and the more the weight loss is significant, the water quantity and the food quantity consumed are falling and for some strains, the general condition is worse. The longer the travel is, the less the rodents are stressed, the more the weight gain is significant, the water quantity and the food quantity consumed are increasing and the general condition of the animals is good! As a conclusion, contrary to current convictions regarding the stress generated by transport, the transport of rodents on large distances benefits the animals compared with a transport with a short duration. The true reason of stress is not the transport, but the change of environment for the rodents.

PE16 Animal by-products for diagnostic, research and educational purposes: a sparkle of light in a complex regulatory framework?

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Belgian Biosafety Professionals.

Activities with animals for experimental purposes are subject to strict regulatory requirements imposed from national and international level. Some are obvious, such as the animal welfare regulation. Others are less known, such as the regulation concerning animal by-products. As animal by-products and their derived products are a potential source of risks to public and animal health, their use, transport and disposal are strictly regulated at EU level (Regulations (EC) 1069/2009 and 142/2011).
Animal by-products (ABP) are defined in Article 3 of Regulation (EC) 1069/2009 as 'entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption'. The ABP regulation covers a wide range of ABP with varying risks (category 1, 2 and 3), and different purposes of use (e.g. animal food, clothing, scientific research). Consequently, not all its requirements apply to all users of ABP, making it sometimes difficult for users to find their way in the complex regulatory framework. Such is the case for ABP intended for diagnostic, scientific research and/or educational activities (e.g. whole animals and parts of animals used for scientific or educational purposes). For the promotion of science and research and to ensure the best possible use of ABP and their derived products in the diagnosis of human and/or animal diseases, the EU therefore authorised its member states to lay down conditions for ABP intended for research, educational and diagnostic purposes. Such conditions may include specific registration procedures that will enable institutes to use ABP for research, educational and diagnostic purposes. Furthermore, they can comprise requirements for transport, processing and final disposal of the ABP. The aim of the poster is to summarize the specific conditions for the use of ABP intended for diagnostic, research and/or educational purposes taking the recent implementation of the EU regulation into the Belgian regulatory framework into account.

PE17 Innovate design of a training microsurgery area for medical and research staff.

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Nowadays, with the implementation of stem cells, growth factors and decellularization processes tissue, microsurgical techniques have increased. However, research projects carried out by medical doctors and scientists in laboratory animals (LA) must meet the 3Rs principles, having to receive prior specialized training. A microsurgery area specially designed is showed to meet standards of quality training in medical specialties with latest technology facilitating the Refinement principle in LA.
Objectives: Design a microsurgical area with the latest techniques and advances according to the Refinement principle (Rp) of the 3Rs in procedures of animal experimental models, while achieving excellence in microsurgical training. Material and methods: The area of microsurgical techniques in the CTF has 10 work stations equipped with a) surgical microscopes; b) worktable with automatic hydraulic regulation and a dual digital screen damage proof; c) cold light source with two outputs and an articulated arm for microscope; d) a pedal milling machine with different milling cutters; e) a capsule with three threaded fasteners for fixing anatomical parts especially designed to comply with the Rp and f) video endoscopy unit with light source.One screen of the workstation shows the surgical field user, while the second screen may shows: 1) surgical fields of another user; 2) the teacher's digital presentation or 3) a desktop PC for the user so practices can be recorded "in situ". The area is also provided with: g) a digital large screen for surgical field projections and exchange of images of any other user, which is controlled from the tutor station server; h) an automatic screen pop-pair and a digital projector presentations; i) an electrical bipolar hemostat with pedal; j) 3 outputs valves for O2 and air are installed with sevoflurane vaporizers providing inhaled anesthesia to all workstations and connection to an anesthetic induction chamber for rodents and k) euthanasia camera with automatic insufflators system with CO2 supplier. Results: The microsurgery area of the CTF has enabled the delivery of residents learning programs (with 10 medical residents per year) and research projects of scientific staff of INIBIC, USC and UAC (a total of 10 groups) optimizing as far as possible the Rp. These facilities have also allowed the holding of microsurgical techniques courses for external and internal staff for different medical specialties with a total of 70 students since its inception. These practices classified "non recovery" and all severe procedures have been supervised every time by technicians and specialized veterinarians of CTF. Discussion and conclusions: CTF's

microsurgical area is emerging as one of the unique designs compared to other similar centers. These high-tech design facilities and allows to develop experimental accreditation, acquisition of microsurgical skills ensuring high quality practices harmonized with the 3Rs and optimizing the Rp effectively.

PE19 Clinical simulation area design in centro tecnológico de formación de la xerencia de gestión integrada de a coruña. Integration of new technologies in the training of professionals. Searching for alternative methods.

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Clinical simulation (CS) in hospitals for medical education has been markedly developed in the last 15 years. In addition, several institutions have decided to centralize CS equipment and management of practices of medical specialization in experimental areas of each center. New veterinary responsibilities (VR) have been assigned to veterinarians belonging to experimental units combining the use of animal model (AM) and CS in the workshops under the Replacement principle of AM (RP).

Objectives

Describe a CS area design and technology equipment for strengthen the RP in training courses for staff of health and State Administration Services and defining VR for veterinarians who perform their actions in hospitals or medical research units

Material and methods

Facilities

The CS area of the CTF consists of a classroom, a room for adult patient (RAP), another room for pediatric patients (RPP) and between both the control room (CR) is located.

Equipment

The RAP is equipped with: a) an adult patient dummy on a mobile stretcher with automatic hydraulic system, b) hi-tech ventilator and advanced monitoring of vital signs, c) defibrillator and anesthesia instrumental, d) storage of medicines and medical consumables, e) material for scenario adaptation and makeup kit for dummy and actors involved, f) 3 cameras recording actions of students in the exercise. These sequences are displayed on monitors located in the CR and g) a large monitor on which students can observe all vital signs scheduled in the dummy during exercise. The RPP is equipped similar to the RAP but with inventory and medical consumables for pediatrics. Similar image and sound as RAP is installed and managed by the CR.

8 monitors showing student's actions inside the RAP and RPP are located in the CR with 2 PCs containing software programming dummies for displaying clinical signs. A Hi-Fi equipment to regulate sounds and move the installed cameras, transmitting the sessions to a large monitor installed in the classroom. Students performing the exercise in the RPP and RAP, are observed by other students that draw critical opinions.

Results

This CS area design has allowed the celebration of 210 simulation workshops with 18 students/course, introducing actions very similar to reality and without using any AM,

In this CS area, VR have consisted of: a) technical coordination of activities of CS training courses and workshops, b) management and preparation of simulation material (SM) and advice on software programming, c) attend as workshop support staff, d) proposals for using SM in those courses where AM can be replaced and for new hi-fi SM acquisitions.

Discussion and conclusions

This innovative design for the CS area of CTF has allowed situations similar to reality and better options than using AM, with the interaction of students among them, with actors and the dummies.

Regarding VR, great skills for management and coordination, extensive prior knowledge in CS and innovative spirit are required.

PE20 EUPRIM-Net – the European virtual primate centre and centre of excellence for the 3Rs

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German Primate Center.

The European Primate Network (EUPRIM-Net) was implemented in 2006 as an Integrating Research Infrastructure. The network connects non-human primate (NHP) centres in Europe and has received more than 11 million EUR of funding for network-, access-, and research activities around NHP keeping, husbandry and research. The network is well established and has become a leading voice in Europe for science that meets the highest ethical standards and has served as important resource for NHP welfare.

Biological and biomedical research ensure good health of humans and animals and this way contribute to a good quality of life. If no alternative exists this research includes animals comprising a small number of non-human primates (NHP). To protect animals used for scientific procedures the European Parliament and the Council of the European Union adopted Directive 86/609/EEC which was revised in 2010 and Directive 2010/63/EU on the protection of animals used for scientific purposes is now in place. To date all EU member states have transposed the Directive into National Law; however, the implementation process is still ongoing.EUPRIM-Net3 proposes activities aimed at helping with the implementation of Directive 2010/63/EU by contributing to and propagating animal welfare and the 3Rs concept of refinement, reduction, and replacement amongst NHP research and testing.EUPRIM-Net³ aims to establish a long term offer to the research community in education and training, exchange platforms and knowledge transfer, expertise with NHP, animal welfare assessment, access to NHP biomaterial, NHP models, and non-invasive imaging with NHP. Moreover, EUPRIM-Net research teams will aim at helping in the selection of individual animals for neuroscience and infection research. Another research team will develop assays that avoid the use of animals in the pre-experimental phase, reducing the number of animals that have to be used. EUPRIM-Net3 will if granted work closely with stakeholders from the industry and non-human primate facilities with smaller numbers of primates, as well as non-European primate centres.

PE21 The in vivo and in vitro experimental laboratory complex

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Faculty of Pharmacy, Medical University of Lodz has a Complex whose function is to conduct experimental studies on animals and cell lines and to breed laboratory animals. The central part of Complex is a modern Animal House, with equipment in accordance with EU Directive 2010/63, fully compatible with the Law on the protection of laboratory animals.
Infrastructure, including operating, metabolic and behavioral equipment ensures the maintenance of animals in the regime for purity sanitary barrier (SPF), allows to keep the animals with reduced immunity (IVC) and on the contained use of GMOs animals. In addition, the complex is registered in the Agency for Restructuring and Modernization of Agriculture and authorized to buy and maintain farm animals. We provide services for the implementation of researches within various fields. We offer help in designing, applying for permission to experiment, executing procedures and elaboration of results. We are constantly expanding our expertise through the diversity of researches. We also train in the laboratory animal procedures and share research equipment to start–up companies and for projects. Our laboratory is covered by the management system according to PN-EN ISO/IEC 17025:2005, including 9001:2008. We offer standardized testing of biological evaluation of medical devices based on BS EN ISO 10993: 10993-2:2006 (animal handling); 10993-3:2009 (genotoxicity, carcinogenicity and reproductive toxicity); 10993-5:2009 (cytotoxicity); 10993-6:2009 (implantation); 10993-10:2011 (irritation, sensitization); 10993-11:2009 (systemic toxicity). In the nearest future there are planned projects related to the implementation of procedures and inter-laboratory comparisons. During implementation, we offer a very favorable price conditions and cordially invite to cooperate.

PE22 When the vegetal makes it possible for microsurgery training in Cambodia, without animal suffering and pain.

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Université Lyon [1] - WASP science.

If it is important to provide a systematic use of replacement in the development of new training programs, it is determined to take account of local conditions and socio-economic environment to sustain it. Thus in Cambodia, a local plant resources, free from health risks, and available at low cost, is a surprising but ultimately decisive substitute in the feasibility of a microsurgical education.
Today it seems difficult in Europe to formalize a consensus between the "whole animal" and "without practical work", it seems essential to associate with specific educational objectives, multiple solutions, credible and sustainable in terms of resources allocated to build new teachings. The difficulty of having a dedicated and trained staff, sanitary pressure of local wildlife, the cost compromise the sustainability of a rodent breeding on European standards, even on a conventional status. The need for skill maintenance for surgeons, combined with the establishment of a microsurgical teaching there, were not enough to justify this option. If almost everything is available in the food markets, to the extent that everything falls in food (pig or chicken vessels, ...), zoonotic health risks are incompatible with a reasonable use for healthcare personnel in contact with patients. A first study based on harvested plants in France and extensively tested in terms of conservation, texture, strength, deformability during suture, allowed to consider the use of local plants. After testing several types of storage available locally and various species, local plant resources, free from health risks, and available at low cost, is ultimately a surprising substitute, but in determining the feasibility of a microsurgical education.

PE23 IDEA; A New insights of experimental animal research building design in Turkiye

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A well-planned, designed, constructed, and properly maintained facility is an important element of good animal care and use, and it facilitates efficient, economical, and safe operation. The design and size of our animal facility depends on the scope of hospital research and training activities (such as thoracic and cardiovascular surgery), the physical relationship to the rest of the institutions. And also the geographic location that 15 minutes far from İstanbul Ataturk International Airport.
Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital is the individual heart hospital at European continent in Turkiye. Istanbul Experimental Animal Research, Development and Training Center (IDEA) is the one of the department of hospital and also in the same campus. IDEA has an effective planning and design includes input from personnel experienced with animal- facility design and operation and from representative users of the proposed facility. IDEA ; has a comfortable technical platform which includes 4 operating room, one angiograpy room, 2 intensive care room, 5 post-operative care unit and also animal breeding floor with technical rooms. Also has histopathology, biochemistry, genetics, cell culture and stem cell laboratories. IDEA; has one training surgery saloon, conference and meeting rooms, library and computer room and also main offices. Most important specialty of IDEA is that has a big botanical garden in the building for personnel and researchers. Personnel can easily see botanical garden while they are working from all labs and operating rooms. For human health safe, for daylight working and stuff welfare IDEA has terrace and special resting area for researchers. All rooms for animal species are separated with barriers and also has noise barrier between the walls. For good animal management and human comfort and health protection require animal facility separated from personnel areas, such as offices and conference rooms. With carefully planning we made it possible to place animal housing areas next to or near research laboratories but separated from them by barriers, such as entry locks, corridors and floors. An animal facility designed and constructed in accord with all applicable state and local building codes.IDEA is the first concept of animal and human welfare together building practice; laboratory animal facility includes rat, rabbit, guinea pig, mice, zebra fish and swine and sheep in the same time in Turkiye.

PE24 Comprehensive Training on zebrafish Danio rerio as a new laboratory animal research model for Sri Lanka

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The zebrafish (Danio rerio) is a freshwater fish native to Sri Lanka. It is well known as an ornamental fish. Sri Lankan researchers were not aware of the scientific importance of this fish as a laboratory animal model and its embryo as a new laboratory research model until it was introduced to Sri Lanka at the 1st Conference of the Sri Lanka Association for Laboratory Animal Science in 2014. Second author as the resource person provided the required basic knowledge on the use of this model (1).
Material and methods: The first author was highly impressed by the transparency of the developing embryo during initial training and was keen in establishing a state-of-the-art zebrafish facility in Sri Lanka. Therefore, a 2-week comprehensive training on zebrafish husbandry and zebrafish embryo as a toxicity testing model was carried out at the zebrafish lab in University of Antwerp, Belgium. This training was possible thanks to the National Science Foundation of Sri Lanka who granted an Overseas Special Training Fellowship. The consumables (testing chemicals, microscopic facilities with recording of images, 16 and 24 well plates for toxicity testing etc) necessary for the training was supplied by the University of Antwerp research grants secured by Prof Knapen, Head/Zebrafish lab. The principal areas covered during this comprehensive training include 1. Operation and daily maintenance of the zebrafish housing facility 2. Daily maintenance and reproduction of zebrafish in practice 3. Standardized morphological scoring of zebrafish embryos and larvaeResultsThere are many research applications of this model. Zebrafish embryo model could be used for acute toxicity testing (2) of different substances such as herbal compounds, cosmetics; other chemicals as per the OECD Test Guideline 236 could be used for acute and chronic toxicity of water samples (3) from the areas where the chronic kidney disease is prevalent in Sri Lanka; and for genetic studies. Once zebrafish lab is established the breeding and use of other vertebrate models could be reduced and it is economically beneficial to the institutions engaged in toxicity studies using other vertebrates. Also zebrafish feed on mosquito larvae and therefore whether this fish could be used for the control of mosquito menace could be explored.Further, usefulness of this model could be taught by us with necessary demonstrations at the Postgraduate Certificate and Diploma Courses in Laboratory Animal Science that we are planning to conduct in collaboration with the Utrecht University, The Netherlands, on annual basis to disseminate information on this model to a wider group of scientists from Sri Lanka and beyond. Conclusion: All these will be achievable targets only if a state-of-the-art zebrafish facility could be established in Sri Lanka by the first author as the first and only certified trainee in the country. This will depend on the availability of much needed funds for the initial establishment of the facility.

PE26 CAL-AQUA – A successful example of aquatic species Laboratory Animal Sciences course

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The Laboratory Animal Sciences (LAS) education is mainly focused in rodents, the most used experimental animal model group worldwide. The increased use of some alternative aquatic model species in research demands the LAS courses to introduce additional modules with aquatic species. The European Commission statistics in the use of animals for scientific purposes registered an increase in the number of aquatic animals (mainly fish) used throughout the last decade.
In order to fulfil the needs of the national and international research community, the Aquatic Animal Facility (BOGA) from Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) has been organising, for the past 5 years, an aquatic species LAS course especially focused in aquatic species – the CAL-AQUA. The research community accepted very well this course which allowed the organisation of 12 editions since 2011. The majority of the editions were held at CIIMAR (Porto) but there were also two editions in Algarve and one in Peniche. Almost 250 students, from different nationalities (Portuguese, Spanish, Italian and German), completed this course successfully. These editions registered a high percentage of senior researchers and PhD and master students. The interest of graduation students was also registered being one of the editions organised specifically for this target audience. The CAL-AQUA was designed in 2011 based on the Federation of European Laboratory Animal Sciences Associations (FELASA) guidelines for education and training of persons carrying out animal experiments (Category B - researchers / technicians). The content includes all the general modules while the modules regarding animals were mainly centred in the use of fish as experimental models. Throughout the 5 years of CAL-AQUA we adapted the syllabus including some modules with other aquatic animal groups, namely amphibians, cephalopods and crustaceans. The new guidelines from European Commission for education and training will be introduced in this course and the new CAL-AQUA format will be presented. The major aim of the new CAL-AQUA will be to train researchers to work with three main groups of animals: fish, amphibians and cephalopods. Since there are plenty of other taxa that are not under the new European Directive because they belong to invertebrates (mostly protostomes), the possibility of including new aquatic animal groups in future course editions will be considered because they also can be extremely useful in several different research areas. The CAL-AQUA reformulation will allow researchers from all Europe to attend a Laboratory Animal Sciences course focused in aquatic animals accordingly to the European Commission guidelines, which allow education and training recognition in all other European countries.

PE27 The dual vocational training in Germany: "Laboratory Animal Technician"

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In addition to the standard dual vocational training form we established a training which is clearly oriented towards our specific needs. Through different training opportunities the lab animal technicians following our training form achieve a balanced training that is getting equivalent to the traditional dual vocational training. To this end, we created different job positions, allowing us to hire people with different professional backgrounds.
Germany has a long-standing tradition in its "dual vocational training" which is unique in the international comparison. This dual vocational training system is based on compulsory schooling, including 13 years of education in general. "Lab animal technician" represents an excellent example for this dual concept in Germany. In general, this education comprises three years of training, split up in a part of vocational training school (1/3 of time, theoretical based) and on-the-job training in a company or institute (2/3 of time, practical based). Additionally, the trainee has to follow comprehensive practical courses, organized by all training companies and institutes together.In spite of the great benefit, the practical as well as

theoretical training of the young trainees of this dual training path uncovers disadvantages too. Indeed, the very strict and regulated curricula of the classical dual vocational education guarantees high quality levels, however, due to the vast restriction (up to 90%) to training with transgenic mice under access restricted SPF conditions, it is at the same time overly time intensive. This negatively affects the capacity for educating trainees to meet the overall high demand for lab animal technicians, and often results in a situation, where the majority of animal facility personnel has other professional backgrounds, such as for example veterinary technical assistant, and will receive an "on-the-job" training.For this reason, our institute established an additional "job training" including a curriculum with more orientation to our individual needs in the Laboratory Animal Science. Through different training opportunities the lab animal technicians following our training form achieve a balanced training that is getting equivalent to the traditional dual vocational training. Here, I would like to give an overview about the general history of the profession "lab animal technician", a presentation of the dual vocational training system in Germany as well as an introduction in the educational training concept we established.

PE28 Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS.de)

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Preventable adverse events (PAE) and critical incidents (CI) occur in scientific laboratory practice, even though this work is carried out according to high standards. Negative experiences, however, often remain unpublished and unmentioned. The objective of CIRS-LAS is to create a database of PAE and CI in order to learn from practice and to avoid them in the future. In accordance to the 3R principle, the portal will therefore improve animal safety and reduce the number of laboratory animals.
Material and Methods: CIRS-LAS portal is based on similar databases in human medicine that allow anonymous reports of critical incidents. These reports can be assorted to various sub-categories applying to laboratory animal science. Further explanations of the incidents, for example descriptions of experimental specifications, possible causes, upload of supporting documents and already proposed conceivable solutions, are furthermore possible. A restricted group of persons (members) is authorised to read and to comment on the database content. Results: CIRS-LAS portal serves as an essential contribution towards the implementation of two of the 3R principles. Anonymously shared negative experiences can have an influence on the experimental setup and execution of new scientific projects. Additionally, the comment section of the collected incidents serves as a basis for fundamental discussions and an exchange of ideas between scientific peers on the portal. Currently, persons from Germany, Austria and Switzerland are registered on the portal. In its current developmental stage the CIRS-LAS is already operating multilingual (English, French and German). We aim towards a wide-spread implementation of the portal in the European laboratory animal science community in order to expand the relations between European networks and to advance animal welfare within laboratory animal science. Discussion and Conclusion: The portal has been created with the intent to enhance the quality of scientific work and to minimise critical incidents in laboratory animal science, not only in the context of breeding and experimental procedures but also within basic research. The CIRS-LAS is a valuable instrument that will enhance the safety of laboratory animals (refinement). In addition, a considerable potential for the reduction of the number of laboratory animals (reduction), through the absence of needless repetitions of unsuccessful experiments that have been documented on the portal, can be expected. It is furthermore conceivable that the implementation of the CIRS-LAS could serve to enhance the trust in laboratory animal science, both of the public and the scientific community.

PE29 AniMatch – An innovative web-based platform to share organs and tissues to sustainably reduce lab animal usage

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Besides the general enhancement of the protection level for animals used in scientific experiments, the European directive 2010/63/EU includes the request that "Member States shall facilitate, where appropriate, the establishment of programmes for the sharing of organs and tissues of animals killed." (Article 18). However, in Germany alone 1.3 Mio animals were killed for scientific purposes, used for organ/tissue collection under anaesthesia or for educational purposes in 2013.
The development and deployment of a web-based platform that enables scientists to connect and share organs and tissue of killed animals would directly address the request of the EU directive as well as exploit the existing potential to reduce animals and save the biological resources that are gained. Therefore, we have developed AniMatch (www. Animatch.eu), an innovative web-based platform that allows scientists to register and publish or search for offers to facilitate the multiple use of killed animals. To publish an offer the providing party has to quote the species, type and if necessary the genetic background as well as the number, age, sex, the organ or tissue that is used for own purposes and the timeframe for the killing. The seeking party can search a list including filters for the species and a geographical radius and request while quoting the number of animals and organs or tissue in need. With completion of the request the contact information is exchanged between both parties who are now able to arrange the details of the transfer. Optimizations of our service have been performed after intensive discussions with animal welfare officers in Berlin. Subsequently, we have implemented two safety barriers in the registration process in order to avoid abuse. The measures include approval of affiliation and account activation by the designated animal welfare officer. Furthermore, we integrated a complex matching system that focuses on the verification of the different microbiological units (hygienic management system) that have to be considered during the sharing process. In addition, besides the general solution we have developed an in-house solution. The platform is now ready to be tested for its feasibility and will be provided to interested institutions in Germany as well as Europe. Besides the moral exculpation of scientists AniMatch provides a cost efficient way to use existing infrastructure and to conserve resources in accordance with reducing lab animal usage. To our knowledge this is the first approach to address the challenge for multiple use of killed animals in science.

PE30 Establishment of an expert working group for livestock animals in bioscientific research: Implementation of the 3R principles-challenges and prospects to increase animal welfare and the experimental outcome

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Livestock animals are of highly relevant in bioscientific research and are frequently used for agricultural, veterinary, basic, safety or translational studies. 1.2 Livestock animals are usually obtained directly from livestock production, combined with a lower homogeneity, animal health and hygienic status. Further challenges are the high requirement on personnel expertise or housing conditions including the amount of space and pathogen infection control.
Material and Methods: In contrast to the principles of the 3 Rs (according to European Union directive 2010/63), standardized guidelines and skilled trained staff members recognizing and assessing pain or exerting techniques in livestock animals are lacking for bioscientific research. These factors may have adverse impact on animal welfare and the experimental outcome of scientific procedures. To overcome this dilemma we established an expert working group in 2015 aiming the refinement and reduction of livestock animal utilization in research. Results So far, the working group consists of a broad scientific network including specialists for livestock animals from Germany, Switzerland and Austria. The network is officially accepted as a working group of the German society of laboratory animal science. Initially, five work packages were developed: WP1: animal acquisition/health, WP2: housing/hygienic strategies-, WP3: experimental design/procedures-, WP4: disposition/tissue allocation, and WP5: education–. WP1 and WP2 focused on identification of relevant pathogens which may originate from supplier facility, to implement a standardized scheme for health and housing certification. WP3 designed definitions for assessments and recommendations of standardized anaesthetic and analgesic strategies. WP4 evaluate possibilities of animal disposition, sharing of organs or tissues at the experimental end-point. In WP5 contents of basic courses (according to FELASA) define the further education programmes for staff member on live stock animal trials. Furthermore, international specified interdisciplinary workshops of the WP topics are scheduled. Additionally, refinement objectives, e.g. environmental enrichment strategies, will be developed further within research projects. Discussion and Conclusion: The lack of standardized guidelines or personnel expertise is relevant for livestock animal welfare and may result in higher mortality rates and affect the validity and reporting of experimental data. Therefore, the implementation of the 3 Rs is represented insufficiently in experimental procedures with these animals. Consequently, our expert group aims to develop standardized guidelines and training courses for researchers to improve animal welfare. Further, our group will improve the welfare of livestock animals used in bioscientific research by continued networking across the European Union.

PE31 What do we know about cephalopods housing? Impact of Directive 2010/63/EU on cephalopod research

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Cephalopods are the sole invertebrates included in Directive 2010/63/EU; requirements for their housing are not included in Annex III. However, indication for cephalopods' care are provided in the Guidelines for the Care and Welfare of Cephalopods in Research (1). We compared information published in scientific papers before (2010) and after EU Directive implementation (2013) to further provide technical data and to monitor the reaction of the scientific community to the new regulatory framework.
DATA SOURCE & METHODOLOGY: We extracted from ISI Web of Science scientific papers published in 2010 and 2013 based on the query 'cephalopod*' (topic field) combined with the list of EU countries (address field). From this dataset (2010: n=65; 2013: n=41) we collected information on technical data included: source of water (natural/artificial seawater), water parameters (e.g. temperature, salinity, pH), water circulation systems (flow-through/recirculating systems), tanks (material, size, colour), illumination regime, number of animals/tank, enrichment.Data were ranked with countries of Institutional affiliations and matched with a previous survey (2) to analyze relationships between species, housing and countries where experiments were carried out . In addition, comparison is made considering years of the study and information included. RESULTSFor all topics considered, we noticed an improvement over years in the description of housing included in papers. For example, the number of papers not including any information on source of water dropped down from 73.8% (2010) to 26.8% (2013). In the 2013 water quality parameters appeared to be described with greater detail (63% vs 80%, 2010 vs 2013). DISCUSSION & CONCLUSIONS: Detailed description for best housing conditions for cephalopods are still to be provided, but after the implementation of Directive 2010/63/EU more data on cephalopods housing have been included in published studies. Increasing the level of detail in methods of any published research favours sharing of best practices and the achievement of comparable results in different laboratories. Standardization of protocols, including housing and care, favours the number of studies to be possible worldwide and it's beneficial not only to researchers, but to everybody involved (e.g. authorizing agencies, veterinarians). The EU scientific community has to refer not only to mandated minima included in regulations and to recommendations, but also to newly established guidelines for reporting animal research such as ARRIVE and GSPC (3), thus interest to these "details" is expected to growth. We believe that improving technical details for housing will also greatly affect animal welfare and 3Rs policy applied to cephalopods, as for other laboratory species. Finally, this collection of information may provide the ground for the implementation of the cephalopod Guidelines, thus facilitating the provision of a tool aimed to prepare better facilities for cephalopods.

PE32 Improving Training in Experimental Design is Essential: Reflections on Delivering Effective Training and Why it is so Important

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Fund for the Replacement of Animals in Medical Experiments (FRAME).

Recent studies highlight deficiencies in the design and presentation of animal experiments, which have consequences for the validity of the resulting data. Directive 2010/63/EU means there is now a legal requirement for training in the design of procedures and projects. Good experimental design is one of the most effective ways to reduce and refine animal procedures. Researchers are not gaining essential training in this area. Reflections on delivering such training and its importance are given.

Under the requirements of Directive 2010/63/EU on the protection of animals used for scientific purposes, researchers must be trained and competent in the requirements of replacement, reduction and refinement and, in design of procedures and projects, where appropriate. Despite this, recent studies such as [1] and [2] have shown that there are significant problems with how animal experiments are designed and reported. These deficiencies can lead to strong bias and negatively impact the validity and rigour of the findings, which in turn raises ethical questions about the appropriateness of the use of animals. This recent literature highlights an important issue that needs much more attention to ensure the numbers of animals in experiments are reduced and that those experiments that unavoidably (at present) use animals provide rigorous high quality results. It also identifies problems with regard to publishing information on experiments and the way research is assessed. In the experience of this author scientists are not necessarily receiving adequate training in experimental design to enable them to identify these problems both when designing experiments and when reviewing them. Good experimental design is one of the most effective and immediate ways to reduce and refine animal procedures, but researchers are not getting sufficient training in this essential area for completing ethical, rigorous and efficient research [3]. Having developed and delivered training in experimental design and statistics it is clear that demand for such courses is high and that skills need improving. Pre and post-course testing indicate that even basic principles are not always understood. Participants report being exposed to new knowledge and show an improvement in their knowledge. This presentation discusses why improving the quantity and quality of training in experimental design is essential with reference to ten years' experience of providing such training.

PF2 Activity and experiences of a legitimate peripheral Animal Welfare Committee in five year overview in Hungary

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The University of Debrecen is the oldest continuously operating institution of higher education in Hungary. The University of Debrecen Committee of Animal Welfare (UDCAW) has been operating since 1999 (formerly, under the name of University of Debrecen Committee of Animal Research). The tasks of UDCAW are established by Ordinance 40/2013 (II. 14.) of the Hungarian Government on behalf of EU Directive 2010/63/EU of the European Parliament and of the Council.
The main goals of Committee are to improve the animal health and welfare in research, to promote the principles of replacement, reduction and refinement (3R) in daily scientific work, to organize lectures and trainings in laboratory animal science, to supervise the animal records and prepare reports, to manage the project authorisation. We would like to show in a five year overview the activity and experience of our legitimate Committee of Animal Welfare, which is one of Hungary's most active committee in this field. In the University more than 100 experiments are coordinated yearly (altogether more than 200 experiments in the aforementioned period). Our study presents statistical data of animals (e.g. number of animals, class of species, purposes of experiments etc.) used for scientific purposes in University of Debrecen, compared with National and with the Member States of the European Union available data from same period.

PF3 Developing a sustainable and impactful 3Rs Recognition Programme in a Global CRO

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The Covance Global 3Rs Working Group (WG) first met in March 2014 with the aim of promoting the 3Rs through a new recognition programme. During the first two years, 37 projects and 116 employees were recognised for their valuable contributions to the 3Rs at Covance. This poster explains how the Working Group was established and its approach to developing and introducing a successful and sustainable recognition programme.
Sponsored by the Head of Early Development and the Vice President of Global Comparative Medicine and Animal Welfare, the WG was formed to promote and share 3Rs developments across Covance through a new global recognition programme. WG members include scientists, veterinarians, operations leaders and animal welfare experts representing all Covance sites where animals are bred or used in research. After bench-marking similar programmes at other research institutions the WG agreed that the Covance programme should: 1) be team oriented; 2) be continual rather than limited to a single annual event; 3) include the wide variety of 3Rs activities across Covance, and 4) be easily accessible to all employees globally. Next, the WG developed an efficient process by which employees can submit their 3Rs developments under one of five categories including; Scientific, Surgical Techniques, Enrichment, Laboratory Animal Care, Methodology and Ideas or Proposals at a proof of concept stage. The WG also developed a process whereby each submission is reviewed against predetermined criteria relating to 3Rs impact. The most impactful submissions in each category are entered for an annual award, publicly recognised via posters, all staff email "flyers" and a globally accessible SharePoint site. All entrants also receive special certificates of commendation personally signed by the programme sponsors. Since the programme's inception, 37 projects and 116 employees have been recognised for their valuable contributions to the 3Rs at Covance. In December 2015, the first Annual Global 3Rs Award was presented to one of the 37 projects deemed to have had the most significant impact on animal welfare. Recipients received commemorative trophies and were featured on the Covance global intranet. This new programme has also been featured in the company's social responsibility report and has received positive feedback from Covance clients and regulators.

PF4 Selection and preparation of beagle dogs for re-homing.

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Selection and preparation of beagle dogs for re-homing
In the last few years, pharmaceutical companies apply a high standard of ethical principles and guidelines regarding the welfare of laboratory animals. Upon these guidelines, Janssen Research and Development introduced a re-homing policy for beagle dogs. The Johnson & Johnson policy on the "humane care and use of laboratory research animals" describes different principles and rules that all J & J companies, including Janssen Pharmaceutica must comply to. Therefore, the J & J companies systematically apply the principle of the 3R's: Replacement, Reduction and Refinement. Focusing on Refinement, it is of Janssen's policy to re-home or relocate animals when they cannot be re-used within an acceptable period of time. In order to properly perform this transition from being a lab animal to being a beloved pet, Janssen Research and Development makes efforts to support the non-profit organization who takes these animals under their care. Our devoted staff members train the dogs on a leash and introduce these animals give them their first steps in the open air. It can be concluded that, the effort Janssen Pharmaceutica makes to prepare the dogs, suitable for re-homing, is in the best interest of these animals and to give them a better start in their future lives. With this program of walking the dogs and policy of re-homing the animals, a high standard of animal welfare is accomplished.

PF5 Soft adaption of laboratory animals to different environmental guidelines.

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Animal husbandry for scientifically studies must be a compromise between laboratory circumstances and animal welfare. Even with great efforts for providing adequate living conditions in laboratory research it should be clear that private housing has to offer better species appropriate living conditions. The resettlement to a new home represents a challenge for the new owner as well for the animal. A crucial point are the animal requirements in the first days.
No empirical data collections are available but we put together our experience on laboratory animals with the experience of a society for laboratory animal release on the resettlement into private homes. Together with the literature, we want to establish a manual for an animal resettlement into private homes. The aim of this study is a guidance of these requirements for a better understanding between animal and owner. As the law

for animal experiments regulates the housing under laboratory conditions, there is a law for the private husbandry. Of course the requested standards for the private home are crucial different compared to them in a laboratory facility. For example it is not allowed to keep rats separated. The cage for two rats must have a size 80x40x50 plus 20% of the ground floor for one more adult rat. This means an eight times bigger cage than under laboratory conditions. Additionally there has to be cage equipment in a three dimensional arrangement. Furthermore rats from laboratories are not habituated to the requested cage enrichment in a private home. Moreover, private feeding differs from the nutrition in laboratory animal facilities. For that and other reasons it was often seen, that replaced animals suffer from the obviously better housing conditions because they are habituated to them. Hence it is in no way a help for the animals and as well for the new owners to replace laboratory animals directly into private homes. Following animals welfare they slowly have to be adjusted to their new home. For example rats should be kept in smaller cages with less enrichment in the first days.Facilities with laboratory animals should not salve their conscience in the resettlement to private homes without any guidance to the new owner!

PF6 Updates to Research in Laboratory Animal Ethics

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This paper is a systematic review of literature on current research in laboratory animal ethics, welfare, and policy. It also provides suggestions and advice for best practices to help ensure and improve animal welfare in China.
Animal experiments are still the preferred method for research on disease etiology, evaluations of drug efficacy, and assessments of drug toxicity and safety in the biomedical field. Due to advancements in modern ethics understanding, the use of laboratory animals is tied with an important ethical debate. Through a systematic literature review, this paper investigates the ethical origins of animal welfare, existing research on animal welfare and the 3R principle (Reduce, Replace, Refine), and the development of the current Laboratory Animal Welfare Act. Based on this review, we identify several issues with the current system, propose some practical suggestions, and offer advice for ensuring and improving animal welfare in research in China.

PF7 ACIS - For in vivo work from experimental design to Home Office Returns

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We will describe our recent adoption of the ACIS system and the functionality therein which coordinates all aspects of in vivo work from experimental design, to the ordering of animals, training status of operators to the fate of the animals and even generating Return of Procedure reports for the Home Office.
2010/63/EU changed legislation thereby passing responsibility for Personal Licence Holders (PILh) carrying out in vivo techniques from the Home Office to the Licenced Establishment. Article 23 of the Directive identifies those persons requiring education and training. Article 24 places greater responsibility on establishments to ensure "staff are adequately educated, trained and competent. Furthermore, it is important that staff are supervised until they have obtained and demonstrated the requisite competence."Animal Care Information System (ACIS by Instem) is a centralised database system for managing all animal related activities in the facility. This covers the entire extent of the interaction between establishments and animals, including · Procurement· Study design and scheduling of activities· Training and competency records – linked to study design; check against operators competencies; reminders for expiry, Continued Professional Development (CPD) points· Recording procedures on animals· AWERB (Animal Welfare and Ethical Review Body)· Fate of animals including severity· Return of procedure dataExisting processes for tracking trainingUCB was already using the ACIS system for procurement, studying tracking and Home Office reporting. UCB had an existing in-house designed database for tracking training, but decided to move this into ACIS with the following advantages:· Cost savings by retiring the existing system· Moving to a more flexible system· Greater security with a full audit trail· Greater growth capacity due to the type of database ACIS runs on· Greater compliance by having training records integrated with study records· Greater visibility with configurable reports and improved searchingThis poster will provide an account of how the change was implemented and identify any areas of subsequent improvement.

PF8 ELLI Record-Keeping System as a Tool for Assessing, Reporting and Enhancing Animal Welfare at the University of Turku Central Animal Laboratory

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Ongoing monitoring of research animals is required for reducing of suffering and improving animal welfare. The communication between researchers and caretakers, who see the research colony daily, is essential to get real-time information on health of the animals. Simple, fast, and efficient tools are needed in order to resolve animals' health issues in animal facilities.
Central Animal Laboratory of University of Turku (UTU-CAL) uses the ELLI record-keeping system for management of animals used in research or teaching. Features in ELLI include maintaining accurate records of the number of animals, their origins and fate, animal orders, animal traffic, work orders, and medical history. Recently, a real-time notification system for recording and alerting observations has been added to the ELLI-system. For assessing the animal welfare, animal caretakers input the clinical observations accompanied with a picture to ELLI. ELLI users get real-time notifications on welfare assessment from a browser-based interface. The system alerts according to a three-tier scale (+++ extremely urgent, ++ very urgent, + urgent) by e-mail and/or SMS. Depending on the urgency, the researcher must react to status of the individual animal within 23, 72 or 120 hours, respectively. The system alerts the researcher until the animal's health problem solved, otherwise UTU-CAL issues an invoice for veterinary visit. Between July and December in 2015, active number of animals in our facilities was 34534, of which 30905 were mice. The number of clinical observation alerts during this time period was related to approximately 1% of rodents in the facilities. Among the clinical observations reported in ELLI, skin problems (wounds, bites) and malformations were over-represented each exceeding 10% of the reported cases. Abscess/tumors, abnormal posture/appearance and changes in coat/skin categories comprised of 3 to 5% of the reported cases. Reproduction, behavior, posture and mobility, tremors, teeth, eyes categories comprised of 1 to 2.5% of the cases. Breathing and vocalizations categories remained under

1% of the reported cases. ELLI, with its new feature, has became a significant tool for facilitating communication between researchers and animal caretakers thus improving animal welfare and reducing suffering. Animal welfare body (researcher, animal care taker and veterinarian) gets valuable information from the notification system that helps directing the efforts to solve any health problems and suggesting improvements by implementing 3Rs.

PF10 Exercise performance tests in mice

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Animals have been a fundamental part of product development across multiple business sectors including cosmetic, drugs and vaccines. Although animal testing is still indispensable in many areas of R&D for new products, in many cases the use of animals in batch release testing is historically driven from previous experiences with the same or related substances.
Animal welfare, economic and scientific perspectives as well as international horizontal legislations to protect animals used for scientific purposes, increase the demand for implementation of alternatives to in-process and end-product testing. Nevertheless, full replacement of animal testing is not a linear process but more often requires a series of smaller investments and a continuum of changes involving refinements, reductions and ultimately replacement(s). The presentation will introduce 3R prioritization algorithm - the multifaceted model of innovation and technology transformation, which intends to provide a framework for the analysis of long-term investments. Based on insights from main variables related to easiness of implementation and business impact, the algorithm offers a modular process for guiding resource investments, tracking progress and identifying risks and benefits for main stakeholders (executive leadership, scientists and non-scientific partners). The model is broadly applicable and can be used in private and/or public organizations and across product sectors. Such an integrative perspective is important in creating the complete picture of the dynamics of system innovations such as transition from historical animal models to the 3R based methodology.

PF11 Scientists' attitudes to animal use and the 3Rs and the influence of LAS training

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Scientists using animals are trusted to not only follow regulations, but also to think and act ethically [1]. Training in lab animal science (LAS) aims to meet these expectations, by promoting best practice and the 3Rs. We surveyed participants in courses following FELASA guidelines (B and C), on attitudes to harm-benefit appraisal and the 3Rs. To assess whether these are affected by training, a first survey (FS, N=310) was carried out before the course and a follow-up (FU, N=200) 6 months later.
On-line questionnaires were sent to participants in LAS courses (2014-15) in four European countries: Portugal (Porto, Braga), Germany (Munich, Heidelberg), Switzerland (Basel, Lausanne, Zurich), and Denmark (Copenhagen). Mean age: 30.2 years (SD=5.7), fem./male: 65/35%. Initial 3Rs awareness (i.e. being able to name them) was 53%, differing significantly between venues (p=.026), and improving to 91% in FU (p<.001). In the FS 47% agreed to "not know as much about the 3Rs as they wanted", dropping to 16% in the FU (p<.001). Course participants valued Refinement more than Replacement and found it more feasible. Of those with animal research experience (67% FS; 81% in FU), only 17% considered some step in their work could potentially be replaced by alternatives, a number unchanged in FU. Moreover, only 18% (FS) considered full-replacement possible in the foreseeable future (no FU dif.). Most (72%) considered that compared to "full-Replacement", "full-Refinement" is more readily feasible (no FU dif.) and more "urgently needed" (FS:69%; FU:74%, p=0.05). When confronted with Reduction-Refinement dilemmas, Refinement was typically favoured: given a "group vs single housing" dilemma, most would rather group-house than using fewer mice (70% in FS, 78% in FU, p<.05); also, 58% would provide Refinement also when affecting research outcome (nesting material to mice models of Huntington's disease, known to delay disease onset) (no FS-FU dif.). Faced with a case-study in which body-temperature was to be recorded by telemetry, most would provide post-operative analgesia after sensor implantation: 61% did not consider drug effects an issue, provided controls were also given analgesics; 16% would relieve pain even if it interfered with data; 12% did not consider analgesics needed for this minor procedure; and for 11% while any pain was likely to be mild, data skewing was an issue (no FS/FU dif.). Respondents were asked to do a harm-benefit appraisal (accept / reject) of three hypothetical research protocols, each with a given species/severity/purpose combination, and variations of these protocols in one or two such factors. Use of dogs or primates led to a higher rejection rate in FS, for all purposes (e.g. study drug addiction, obesity or drug development for leukaemia) and severity combinations. Overall, courses improve knowledge of 3Rs. Unsurprisingly, they also strengthen scientists already high focus on Refinement, whereas focus on Replacement remains low.

PF14 Animal Ethics in Animal Research: a comprehensive European book on animal research ethics

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With the implementation of Directive 2010/63/EU, many more professionals need insight into animal research ethics. The obligatory training to obtain permission to work with research animals is now required to include ethics, and every institution is required to have an institutional body to oversee 3Rs and other ethically relevant issues. However, comprehensive text resources have been missing.
We are presenting the book Animal Ethics in Animal Research, to be published by Springer in 2016 as the first comprehensive European text book on animal research ethics. This book aims to help the reader see the values embedded in the discussions of animal use in research, the complexity and inter-relatedness of the many issues, and to show the practical context and usefulness of ethical discussions of animal experimentation. The book contains seven chapters that cover different aspects of the practical, ethical and legal issues connected to research animals. Chapter 1 describes issues related to research ethics in general and to designing animal research especially, focusing on the difficult challenge to balance an objective scientific approach with an empathic relation to the animals used in research. Chapter 2 discusses what "good scientific practice" means for research with animals, focusing on the concept of animal welfare and how it can be applied, classification of degree of severity as well

as the 3Rs. In chapter 3 we present the most relevant normative ethical theories and also discuss their stance on use of animals in research. This chapter is closely linked to chapter 4 where six recent examples of animal based research are presented, scrutinized from the position of the ethical theories, and finally the role of social aspects for evaluation of research is discussed. Chapter 5 differs somewhat from other chapters not only by its length, but also by holding less of discussion in favour of presenting a state of the art of legislation in many parts of the world. The main focus is on EU and the recent directive, but also includes descriptions of ethical assessment and legislation in Asia, Australia, North America and Latin America followed by presentation of a number of guidelines developed by the research community itself. As legislation is strongly influenced by public concerns regarding animal research, we focus on public involvement in chapter 6. After exploring public attitudes and strength of engagement with animal research, the chapter ends with an elaboration of the democratic aspects of public involvement, as this is crucial for public support. Finally Chapter 7 tries to look into the crystal ball and provide a guesstimate on where both the use of research animals and the ethical discussions connected to it may go in the future.

PF15 Strong and weak voices in sensemaking processes: Scientists' social networks in moral decision-making

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Making sense of ethical and moral issues is a social process. Individuals do not make sense of these issues on their own but rather observe the attitudes and behaviours of others as well as consult with others before making decisions. We adopted this perspective to the setting of in-vivo animal science and addressed the question of who is (not) consulted in the process of sensemaking regarding topics that potentially raise ethical issues for scientists.
Data for this paper was gathered by means of a survey, which was distributed to a random sample of 1164 biomedical scientists in the UK who use animals in their research. The response rate was 37%. We asked the respondents to give information (i.e. occupational role, topic of discussion) about the individuals with whom they had spoken about animal care and welfare in their scientific research over the last month. Respondents could indicate for each of their relations whether experimental design, operational matters, general moral reflections and/or regulatory issues was the focus of the conversation. The personal networks constructed through this question were analysed using multilevel logistic regression [1]. For methodological reasons, the analysis is based on only those individuals that reported at least one relation. All models have 517 units on level 1 (i.e. relationship) and 149 units on level 2 (i.e. individuals). Results: The results showed that the type of issue discussed depends on whom one talks to. Operational issues are discussed more often with technical support staff, while experimental design and moral issues are more often discussed with another scientist. Discussion and conclusion: It can be concluded that scientists consult others when confronted with ethical issues, but that their discussion partner depends on the issue at hand. While sensemaking around operational issues is shaped in dialogue between scientists and support staff, experimental design and moral issues are discussed mainly amongst scientists. It is not surprising that scientists would consult technical staff on operational issues, and other scientists on experimental design. But it is striking that scientists seek out their peers in addressing moral issues specifically, particularly given that technical staff are the "experts" in animal wellbeing. These hierarchical relations could have implications for the outcome of ethical decision-making. This finding also raises the question of what experimental design might look like if technicians' voices were sought. By uncovering the roles of scientists and technical staff in decision making processes related to ethical issues, it becomes clear that ethical decisions regarding animals in science are social rather than individual processes.

PF16 SPERA reasons for research: the motives of scientific research to all citizens

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SPERA-Reasons for research.
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In Italy, scientific research is often perceived as a theoretical activity with no application to everyday life - a reason for this is the lack of a clear and correct scientific elucidation. The public opinion seems to be confused about scientific research - particularly by some aspects of it, such as animal experimentation, which is frequently misjudged and misrepresented in the media. This whole situation can seriously damage research itself and its applications.
During the heated debate regarding animal experimentation that started when Italy was to implement the European directive (2010/63/EU) with the Italian law (DLgs 26/2014), the voice of researchers seemed to be too weak to be heard and add to the debate. That is why in October 2013 AISAL, the Italian Association for Laboratory Animal Science, launched the project "SPERA – Sperimentare per curare", in order to provide accurate and understandable information regarding the 'hot topic' of animal experimentation. In time, through networking among researchers coming from different experiences, SPERA has evolved into a Federation of Scientific Associations operating in different fields. Therefore the name has been changed into "SPERA – Le ragioni della ricerca" because its aim is now broader: explaining the motives of scientific research to all citizens, adults as well as children. Meanwhile, SPERA continues to represent those who are committed to the proper use of laboratory animals in scientific research, so it aims to give to the ultimate beneficiaries of biomedical research the tools necessary to develop a personal critical opinion on this subject, by encouraging an open dialogue and debate that will not resort to sensationalism and prejudice. However, SPERA's main goal is to further the idea that scientific research is a fundamental value for the wellbeing of humankind. For this reason, it intends to promote curiosity among the people by making that of the researcher a less abstract figure in the collective imagination. Our main tool is the direct contact with new generations, bringing into the schools the direct experience of scientists, avoiding the "interpretation" of scientific results, usually obtained through the media. So far 6 schools in the city of Rome have already participated to the pilot projects regarding subjects such as neurosciences and the environment (with about 300 students from elementary schools, 100 from middle schools and 800 from high schools). Our high school experience was particularly promising: although the topics we presented were complex and delicate - the scientific method, the researcher's tools (including laboratory animals), vaccines and biotechnologies – and some of the students were already strongly opinionated about it, they proved to be interested in broadening their knowledge and in participating in an open and respectful debate with more correct information at their disposal.

PF17 Progress towards ending severe suffering: working together to achieve positive change

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All laboratory animal suffering is a concern, but the RSPCA believes that ending severe suffering should be a top priority. There are a number of reasons to do this: (i) the ethical benefit of reducing suffering, (ii) the legal requirement to minimise suffering set out in Directive 2010/63 EU and (iii) the scientific benefits – it is widely acknowledged that good science goes hand in hand with good welfare.
As a scientific animal welfare organisation the RSPCA has established an integrated programme of work aimed at reducing and ultimately ending severe suffering. Our approach is well supported by the scientific community and has also been endorsed by the UK Government, which cited the project as part of its recent Delivery Plan on animal experiments. We have initiated a number of parallel activities including:· A 'Road Map' towards ending severe suffering, outlining the generic key questions and practical considerations that establishments can address in order to reduce suffering for all animals and work towards ending severe suffering. We are actively promoting this throughout 2016 and working with scientists who wish to adopt it. · A comprehensive web resource for the research community, providing guidance and resources to help end severe suffering: www. Rspca.org.uk/severesuffering· A series of projects to reduce suffering within specific procedures or 'models'. We form 'Expert Working Groups' of scientists, vets, animal technologists, animal welfare experts and representatives from the UK regulatory authority, who focus on a particular area of research and see what can be done to avoid using animals or, if this is currently impossible, to significantly reduce suffering. We have produced four reports on reducing suffering in epilepsy, multiple sclerosis, sepsis and rheumatoid arthritis research. A report on spinal cord injury is in progress. This talk will set out how we have been able to work constructively with the scientific community and UK regulator, and provide more information on the RSPCA's resources on severe suffering.

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