Theme VIII – Refinement and Welfare

Coordinators
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Mark Prescott, NC3Rs, London, UK
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Session VIII-1a: Non-human primate use and welfare

Co-chairs
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Session VIII-1a: Oral presentations

VIII-1a-098

Can facial expression identify pain responses in rhesus macaques (*Macaca mulatta*)?

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Successful captive animal management is dependent on addressing welfare needs, including the recognition and alleviation of pain. Facial behaviour is an effective method for identifying pain in humans and rodents, but its use is yet to be evaluated in rhesus macaques. We utilised the Macaque Facial Action Coding System to determine whether facial action units differ in the pain and non-pain state in this species. Video footage was collected opportunistically from animals undergoing potentially painful research procedures and coded for facial movements and general behaviour in four conditions: pre-procedure, post-procedure, pre-analgesia and post-analgesia. Facial action units were analysed using a discriminant functions analysis to identify dimensions that map closely with expected levels of pain. Two dimensions were identified – the first fit closely with predicted pain, and the second was likely associated with arousal. Several behaviours were affected by study condition including cage manipulation, focused vigilance, and bipedal standing, however facial movement mapped more closely with expected pain levels than did general behaviour. While this study is the pilot of on-going research, we conclude facial expressions are useful indicators of pain states in rhesus macaques, and training staff to recognise facial expressions would promote Refinements in husbandry and veterinary care.

VIII-1a-428

Refining the use of non-human primates in research: the NC3Rs as a catalyst for change

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For 10 years the NC3Rs has led the application of the 3Rs to non-human primate (NHP) research. Working with the pharmaceutical industry, we have identified opportunities to reduce NHP use in studies of abuse potential, assessment of candidate therapeutics, and testing of monoclonal antibodies. Our guidelines on NHP accommodation, care and use, adopted by the major UK public funders of bioscience, and our involvement in the funders peer review processes, have raised animal welfare standards internationally. We have invested £1.8M in research and training to refine techniques used in behavioural neuroscience and to develop better methods for assessing pain and distress in NHPs. Working with scientists, veterinarians and animal technicians, we have published in the scientific literature detailed guidance on refining many aspects of NHP care and use (e.g., weaning, transport, training, food and fluid control protocols, rehoming). We have developed an interactive web resource on marmoset care. Our annual Primate Welfare Meeting continues to be a key event for showcasing the latest research in NHP welfare and sharing of best practice. This presentation will review advances in refinement made as a result of our investment and collaborative work, and identify areas where further research and changes in practice are required.

VIII-1a-551

Training primates through the supply chain from breeder to laboratory

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It is well established that the application of positive reinforcement training (PRT) can do much to reduce the impact of scientific and husbandry procedures on research animals including non-human primates. For long-term studies the time investment in training is easily accommodated, but short-term studies (e.g., toxicology) may only last a few weeks and training would add disproportionately to the total study time and hence the accumulation of contingent suffering. At Bioculture, where we breed long-tailed macaques (*Macaca fascicularis*) of the highest health and welfare standard for use in research we began an extensive and intensive programme of PRT across our 18,000 animals in 2009. We aim to train animals as preparation both for general aspects of laboratory life as well as to meet specific requests of end-users. We believe that incorporating PRT in our routine activities helps *Refine* animal health and husbandry procedures, but it also helps researchers in efforts to minimise costs to the animal across its whole lifetime, when addressing the harm:benefit analysis of their

programme of research. This presentation will outline the Bioculture training programme and address challenges and successes through the illustration of results of a series of training studies.

VIII-1a-761

Development of resources for refining research primate use: APV primate retirement guidelines

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The Association of Primate Veterinarians (APV) plays a key role in refining primate use and enhancing primate welfare by developing, educating, and disseminating best practices for nonhuman primate care and management. APV has developed and published a number of guideline documents to assist veterinarians, researchers, and animal ethics committees to enhance animal well-being. Subcommittees of subject experts from within and outside of APV develop draft guidelines. Guidelines undergo several rounds of peer and expert review prior to being adopted but are considered living documents subject to updates, as new information becomes available. To date, completed documents include food restriction guidelines, humane endpoint guidelines, social housing guidelines, and jacket use guidelines. Other documents under development include guidelines for fluid regulation, blood collection, restraint, cranial implant care, female reproductive laparoscopic surgery**,** male reproduction (semen collection), and infusion administration. A recent project has involved developing draft guidelines to assist facilities interested in permanently retiring primates from research use. These guidelines cover selection of the prospective retirement facility, developing an institutional process, preparation of the animal, and preparing the staff and research team for primate retirement. Consideration of these issues when retiring research primates will be addressed.

VIII-1a-897

Nonhuman primate use in North America: overview, trends and important considerations for the future

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The use of non-human primates in North American laboratories will be reviewed and discussed. Invasive studies on chimpanzees are finally coming to an end but the use of other non-human primates has increased over the past fifteen years despite increasing questioning about the ethics of such use. Statistics available for Canada and the United States since 1999 show significant increases in the number of primates in laboratories (both used and held or bred for research) as well as the number of NHPs imported to the U.S. for research and breeding. In addition to a discussion of the overall statistical trends in NHP use, we will: (1) give an overview of NHP use and funding at the eight National Primate Research Centers in the U.S. (which are among the largest NHP labs in the US and house approximately 25% of the total US NHP lab population); (2) explore the reasons behind the end of chimpanzee use worldwide and what that means in terms of a critical examination of the use of other NHPs; and, (3) discuss where efforts should be focused to increase humane treatment of NHPs and decrease their numbers in laboratories.

Session VIII-1b: Non-human primate use and welfare

Co-chairs
Jaco Bakker, BPRC, The Netherlands
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Session VIII-1b: Oral presentations

VIII-1b-092

Refinement of non-human primate disease models and procedures at the BPRC

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Non-human primates are still needed in research on serious human life-threatening diseases. We also have the moral obligation to treat these animals in the best way as possible. The BPRC is involved in non-human primate studies but has also implemented an active and expanding programme in accordance with the principles of reduction, refinement, and replacement in non-human primate disease models. Besides 3R-research on alternatives, the BPRC also focuses on refinement with respect to animal husbandry and care. New housing concepts, positive reinforcement training of the animals and veterinary practices have reduced stress associated with routine husbandry and with experimental studies. Extensive environmental enrichment programs in both breeding and experimental facilities have been developed and implemented. New techniques like telemetry and non-invasive imaging methods are essential to further reduce the number of animals in studies. Continuous training of staff ensures ongoing improvement. Several examples of refinement involving non-human primate disease models, housing conditions and PRT-training procedures at the BPRC will be discussed. The new developments at the BPRC over the past years have enhanced the well-being of these highly complex and social animals.

VIII-1b-126

A holistic approach to refinement improves medical management and safety in nonhuman primate disease models

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Nonhuman primates (NHPs) are used only in experiments when there are no suitable alternative methods or species. Their biological complexity closely mimics the human clinical patient adding unique translational value especially in infectious disease, brain injury, and immunology. The therapeutic need is high for clinical patients in these categories who experience a serious reduction in measured quality of life parameters. Likewise, it's logical to assume there is a substantial burden on the animal considering disease state and management plus necessary monitoring for safety and efficacy trials. The application of refinement can shift the harm-to-benefit ratio, limit experimentally induced complications, and avoid additive effects. We present an overview of our experience with refinement in the complicated NHP model of diabetes including cooperative handling (training), enrichment, minimally invasive instrumentation, and advanced clinical care strategies. The impact of these techniques on morbidity and mortality led to a reduction in overall animals used, illustrating also the interplay between the 3Rs. The scientific value was clearly demonstrated by a significant reduction in confounding in stress sensitive parameters. This holistic refinement approach unequivocally benefits our animals and can enhance predictive value of the model, presenting an opportunity to engage scientists in a meaningful way with the 3Rs.

VIII-1b-143

The use of advanced imaging to refine vaccine and therapeutic studies with non human primates

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Following successful quantification of disease using MR scanning of TB infected lungs (Sharpe et al, 2009, 2010) and in-life proof of principle studies using MRI and CT scanning, macaques infected with tuberculosis by aerosol challenge have been successfully scanned using a mobile high containment pod to avoid contamination of the scanners.

Scans have successfully identified lesions at very early stages of disease before onset of clinical signs and before disease detection by conventional X-radiography. Results of these early infection, low challenge dose studies have ethical implications in refinement and reduction that come from the ability to detect early stage disease following administration of realistic low doses of the pathogen. Use of in-life scanning allows assessment of vaccine or therapeutic efficacy without the need to progress to stages of disease when clinical signs such as cough and weight loss become apparent. The use of scanning allows studies that can define progression of disease and spread to other organs such as the spleen, liver or kidneys in individual animals without the need for serial sacrifice of greater numbers of animals. The use of this technology will allow refinement and reduction in any infectious disease model requiring the use of non-human primates.

VIII-1b-150

Simultaneous PK/PD: automated blood sampling and CV telemetry in conscious nonhuman primates

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Preclinical cardiovascular safety studies normally entail the use of radiotelemetry in large animals such as nonhuman primates (NHPs), while keeping animal welfare a priority. Challenges with the use of NHPs include signal interference during procedures requiring animal manipulation and increases in stress parameters. To address these issues and improve animal welfare, we conducted a proof of concept study whereby automated blood sampling (Culex-L) was coupled with digital radiotelemetry (DSI). Improved sensitivity from this combination system allowed for a reduction in animals and improved correlation between the pharmacokinetics and pharmacodynamics (PK/PD). Four female NHPs implanted with telemetry devices and intracarotid catheters were dosed with moxifloxacin (Moxi), physiological parameters recorded, and blood sampled either manually (Man) or via the Culex-L. During sampling, plasma was collected several times between pre-dose and 24 hours post-dose. Challenges encountered included missed plasma samples and occasional telemetry data dropout. These challenges were resolved by revising component connections and by adjusting tranceiver placement. This study established the feasibility of collecting simultaneous blood samples by the automated Culex-L system while recording physiological measurements via radiotelemetry in unrestrained NHPs. This enhancement in methodology resulted in refining the study while reducing the number of animals involved and therefore, improving animal welfare.

VIII-1b-467 *

Refinement of non-human primate studies by development of adjuvants with less adverse effects

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Adjuvants are additives that are used in vaccines to enhance immune responses directed against pathogens or in experimental animal models for, e.g., human auto-immune diseases. However, many potent adjuvants cause adverse effects. Most notable is the development of granulomatous skin lesions, causing various degrees of discomfort to non-human primates in biomedical experiments. There is therefore an urgent need for new and safe adjuvants. Recently it has become clear that the immune-enhancing effects of adjuvants can be attributed to activation of innate immune receptors such as Toll-like and NOD-like receptors. Based on this knowledge we have engineered a series of luminescent cell lines and used these bioassays to screen new adjuvant candidates for their immune-enhancing effects. In addition, we have developed a 3D *in vitro* granuloma assay to screen adjuvant candidates for their potential to cause adverse effects. We will present data demonstrating that combining these assays as a robust integrated test system can rapidly generate detailed information aiding the development of new potent adjuvants with minimal adverse effects. We will

also show *in vivo* data how these new adjuvants can contribute to the refinement of non-human primate studies and animal welfare.

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VIII-1b -471

Workshop report: 'Alternative methods for the use of non-human primates in biomedical research'

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The experimental use of non-human primates (NHP) in Europe is tightly regulated and is only permitted when there are no alternatives available. As a result, NHP are most often used in late, pre-clinical phases of biomedical research. Although the impetus for scientists, politicians and the general public to replace, reduce and refine NHP in biomedical research is strong, the development of 3R technology for NHP thus poses specific challenges. Last spring, a workshop on "Alternative methods for the use of NHP in biomedical research" was organized within the international exchange program of EUPRIM-Net II, a European infrastructure initiative that links biomedical primate research centers.

The workshop included lectures by key scientists in the field of alternatives as well as by experts from governmental and non-governmental organizations. Furthermore, parallel sessions were organized to stimulate discussion on the challenges for advancing the use of alternative methods for NHP. Subgroups voted on four statements and together composed a list with opportunities and priorities. I will present a summary of the voting and discussion sessions and end with recommendations on 3Rs development for NHP specifically. These include technical, conceptual as well as political topics.

Session VIII-1: Poster presentations

VIII-1-359

European Primate Network EUPRIM-Net II: advancing 3Rs and international standards in biological and biomedical research

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Biological and biomedical research is critical for our understanding of human and animal physiology and medical progress. Where no alternatives exist, this research necessitates the use of non-human primates (NHPs) for the foreseeable future. The EU-funded research infrastructure project EUPRIM-Net brings together ten European publicly-funded primate centers, to promote highest standards using these animals for top-level research. Under the project, the primate centres' infrastructures and expertise are integrated to provide critical services, training and advice to scientific institutions within and outside Europe conducting primate research and to zoological gardens keeping primates. The activities are divided into Network-, Access- and Research Activities all aimed at promoting animal welfare and the 3Rs. Directive 2010/63/EC foresees various animal protection and welfare measures reflected in EUPRIM-Net's activities. The Network Activities are about Education and Training, Best Practice and Veterinary Care, Positive Reinforcement Training (PRT) and Animal Behavioural Management. These activities are supported by Research Activities on Diagnostics and Diseases, Telemetry, and Alternative Methods. Moreover, EUPRIM-Net offers – via an easy online system (http://www.euprim-net.eu)- access to primate material (BioBank) and to primate-based animal models of severe human diseases (PRIMOCID) thus contributing to improving the 3Rs.

VIII-1-360

A cage-based behavioural testing system for NHP – Towards a cognitive neuroscience without restrained animals

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In conventional setups for experiments in cognitive neuroscience, the animals are usually restricted in several ways. For monkeys, movement restriction of the head is common practice when recording neuronal activity by electrophysiological or imaging techniques. However, technological progress especially of wireless techniques allows for recordings without major movement restrictions. We developed a cage-based system monkeys can interact with in an unrestrained and self-paced manner. It utilises a computer controlled touch screen and rewarding system, allowing for setting up various cognitive tasks and recording behavioural data whilst a monkey is interacting with the system. A future addition of miniaturised wireless devices for neuronal recordings, might enable cognitive neuroscientific experiments on unrestrained, freely moving monkeys not separated from their social group. We intend to include an identification system in order to individually assign cognitive tasks depending on the monkey that approaches the device. These refinements of classical setups might result in less stress for the animals and a positive impact on their learning progress. First experiences support the notion that with this system the natural curiosity of monkeys can be stimulated just as if it is part of the environmental enrichment.

VIII-1-408

Using an automated system of positive reinforcement training to refine the process of behavioural training and reduce the need for food control in rhesus macaques

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Behavioural neuroscience research requires subjects 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 an automated positive reinforcement training system (a button press task with variable levels of difficulty using a fluid reward) at the breeding facility Centre for Macaques (CfM) and Newcastle University 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 cage 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.

Preliminary evidence suggests that animals that acquired the task at CfM subsequently performed better in early sessions at Newcastle University. Therefore, it may be possible to use the automated system at CfM to pre-screen animals for suitability for behavioural neuroscience research, thus potentially reducing animal numbers required for studies, refining training protocols and minimising requirements for food/fluid control.

Work supported by the NC3Rs.

VIII-1-681

Optimisation of preclinical use of Non-human primates (NHP) by species-specific Monocyte activation test (MAT)

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Pyrogenicity (fever induction) might be due to drug contamination or product characteristics (immune activation). Vaccines are a typical case for drugs with immune-stimulating potential. The platform technologies (combining known antigens with new adjuvants) increase the speed of vaccine development, accelerated and predictive safety tests are needed.

Non-human primates (NHP) are frequently chosen as a preclinical model (test species) for drug candidate testing before the first application in humans. The whole blood of NHP is typically investigated for biomarkers. In this study we compared four NHP-species to humans *in vitro* by species-specific MAT-versions with fresh whole blood. Compared to each other the NHP-species exhibit a different reaction pattern towards LPS (Endotoxin), but to some extend comparable to humans (depending on the chosen readout). We conclude that NHP-specific MAT are suitable for a first assessment of pyrogenicity of drug candidates or new platforms in the preclinical phase. The use of NHP-fresh blood for *in vitro* experimentation might lead to a diminished (Reduce) and optimised (Refine) use of NHP in preclinical testing. A complete replacement of animal tests in the preclinical phase seems unlikely in the near future.

Session VIII-2a: Best practice welfare approaches – Mouse

Co-chairs
Robert Hubrecht, Universities Fed. Animal Welfare, UK
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Session VIII-2a: Oral presentations

VIII-2a-028

Contribution of animal care and welfare to 3Rs in the pharmaceutical industry

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Animals continue to play a small but vital role in the discovery and development of new medicines and vaccines. We use non-animal method when available, but there is no current replacement for a whole living organism; it is vital to understand how a medicine will interact with our internal system, whether it will be effective and whether it is safe. The provision of healthy, well cared for animals is essential for success of these studies, but we go beyond the basic requirements. In addition to ensuring that our animals receive appropriate housing, food and water we also strive to better understand their needs, to refine their care and environment and hence improve their well being.

This presentation discusses some of the welfare initiatives and resulting refinements in care and consequent changes to the scientific

studies achieved at GlaxoSmithKline over the past few years.

These initiatives include changes to housing paradigms, refinement of experimental techniques, reward systems for new ideas and setting up a global community of practice to discuss and effectively disseminate refinements to animal care. All of this operates within a governance system which ensures implementation of the GSK core principles worldwide.

VIII-2a-284

Refinement and experimental design in publications: a longitudinal case study of ALS research

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Amyotrophic Lateral Sclerosis (ALS) is a human neurodegenerative disease of rapid progression for which no effective treatment exists, resulting in progressive paralysis and death. Similar clinical signs cause distress to research animals modelling the disease. From a 3Rs perspective it is desirable to implement refinement measures such as humane endpoints and housing adaptations.

To evaluate whether measures of refinement and experimental design optimisation were reported, published studies on murine ALS models were analysed (n=267) (Franco and Olsson, 2012). Studies were classified according to a scale of severity, based on the disease stage that animals reached. Studies published in 2005, 2009 and 2011 were included to cover the period in which guidelines to improve preclinical ALS research were introduced (Ludolph et al., 2007, 2010).

Report of compliance with regulation for animal care and use increased over the years reviewed (p<0.01), however the severity level remained unchanged (62% of studies implemented a late stage endpoint). Preclinical studies were reported with significantly higher level of detail than proof-of-concept studies throughout the years. Compared with Huntington's disease, a similar neurodegenerative disease model for which no specific research guidelines exist, ALS research used less severe endpoints with humane endpoints reported in 90% of publications.

References

Franco, N. H. and Olsson, I. A. (2012). *Altern Lab Anim 40*, 271-283.

Ludolph, A. C., Bendotti, C., Blaugrund, E. et al. (2007). *Amyotroph Lateral Scler 8*, 217-223.

Ludolph, A. C., Bendotti, C., Blaugrund, E. et al. (2010). *Amyotroph Lateral Scler 11*, 38-45.

VIII-2a-666

Welfare in the research environment: is what we have as good as it gets?

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Ensuring high standards of welfare for animals kept and used for research purposes requires a multifaceted approach that addresses the animal, its environment and husbandry and research procedures. The environment must meet the animals' needs in terms of appropriate environmental controls, and housing provisions including enrichment where this is appropriate. The animal itself may also be modified to improve welfare, by selection for strains/breeds that do well in such conditions; by ensuring appropriate conditions during development and, where necessary, provision of training and habituation regimes. Procedures and techniques can also be refined to minimize stressful events such as capture and restraint. Nonetheless, there are still many gaps in our knowledge. The principle of enrichment is, for example, widely accepted, but the implications of strain, age, stocking density, environmental changes, and the special needs of animals under procedures are under-researched and the requirements of some groups such as fish and cephalopods have received little attention. High quality rather than *ad hoc* research is necessary for informative results and to provide credibility to the field. Best practice is most likely to be achieved when there are good links between those who use animals in research and the animal welfare science community.

VIII-2a-675

Social housing as the norm for social species of laboratory animals

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The most recent guidelines in 8th Edition of the *Guide for the Care and Use of Laboratory Animals* in the US (NRC, 2011) and the EU Directive 2010/63/EU (EU, 2010) specify that single housing of social species (i.e., most laboratory animals including rodents and rabbits) should be the exception, and this should only occur for special experimental circumstances or when an animal is aggressive and/or incompatible with other animals and when this is done, it should be for the shortest duration possible. The recognition of the social needs of animals in legislation was an important step in promoting animal welfare for laboratory species. However, the challenges of implementing these guidelines can be formidable without basic knowledge of the animals' normal behavioral needs, ability to recognize abnormal behavior and training to implement behavioral interventions. In 2013 a workshop on social housing was coordinated by CAAT, USDA and NIH/OLAW to examine some of the obstacles to social housing with the intent of addressing common issues encountered in achieving the goals of social housing. This presentation will focus on some of the issues discussed and consensuses reached.

References

EU – European Union (2010). Directive 2010/63: Legislation for the protection of animals used for scientific purposes. http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

NRC – National Research Council (2011). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press.

VIII-2a-804

PhenoWorld: increased welfare in a new paradigm for housing and evaluation of rodent behaviour

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Laboratory environment in which rats are housed contribute for an impoverished ethological evaluation and reduced welfare of the animals. We used a new concept for housing and behavioural analysing rodents – the "Phenoworld" (PhW), in which groups of 6 rats lived in a socially and physically enriched environment, and had their feeding, locomotor activity, sleeping and social behaviour automatically monitored.

Home-cage behaviour analysis of animals housed in the PhW, as compared with standard housing revealed the new housing system promotes increased welfare as shown by the increased levels of sleep in the resting phase of the light/dark cycle and by the performance of species specific behaviours such as hopping and climbing. Sucrose preference test revealed an increased hedonism and forced swimming test indicated lower immobility time for PhW living animals, proving an increased well-being of those animals. Light/Dark test revealed that PhW living animals were less anxious than standard housed animals. We have shown that this new ethological refined/enriched paradigm – the PhenoWorld – provides an improved welfare condition for rodents compared to standard cages.

Session VIII-2b: Best practice welfare approaches – Mouse

Co-chairs
Michael Walker, University of Guelph, Canada
Hanno Würbel, University of Bern, Switzerland

Session VIII-2b: Oral presentations

VIII-2b-016

Burrowing and nest building behavior as indicators of well-being in laboratory mice

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The assessment of pain, distress and suffering, as well as evaluation of the efficacy of stress-reduction strategies, is crucial in animal experimentation but can be challenging in laboratory mice. Nest building and burrowing performance, observed in the home cage, have proved to be valuable and easy-to-use tools to assess brain damage or malfunction as well as neurodegenerative diseases. Both behaviors are used as parameters in models of psychiatric disorders or to monitor sickness behavior following infection. Their use has been proposed in more realistic and clinically relevant preclinical models of disease, and reduction of these behaviors seems to be especially useful as an early sign of dysfunction and to monitor disease progression. Finally, both behaviors are reduced by pain and stress. Therefore, in combination with specific disease markers, changes in nest building and burrowing performance may help provide a global picture of a mouse`s state, and thus aid monitoring to ensure well-being in animal experimentation.

VIII-2b-069

Improving laboratory mouse welfare and reducing animal numbers through mixed-strain housing

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All common identification methods for mice can impair animal welfare (Dahlborn et al., 2013) and many experiments utilize homogenous populations that inadvertently contribute to low external validity and poor reproducibility (Wuerbel, 2000). This work aimed to validate mixed-strain housing as a way to remove the need for marking; heterogenize the study population; and utilize a more statistically powerful experimental design. We raised 3-4 week old female C57BL/6, DBA/2, and BALB/c mice in single-strain or mixed-strain trios. At 3-5 months of age mice were assessed for 26 different variables spanning behaviour (e.g., stereotypies), physiology (e.g., blood glucose), and haematology (e.g., white blood cell counts). Single- and mixed-strain housed mice did not differ in any measured variable. Several strain differences emerged (e.g., faecal corticosterone metabolites): all were in the expected direction (e.g., Harizi et al., 2007). Furthermore, there were no interaction effects between strain and whether mice were in single- or mixed-strain trios. Mixed-strain housing also reduced inter-individual variation across all variables (p=0.0012). Mixed strain housing did not modify strain-typical phenotypes nor introduce any

new welfare concerns, thus is therefore a potentially valid experimental paradigm to improve welfare and reduce animal numbers by improving statistical power and increasing external validity.

References

Dahlborn, K., Bugnon, P., Nevalainen, T. et al. (2013). *Lab Anim 47*, 2-11.

Harizi, H., Homo-Delarche, F., Amrani, A. et al. (2007). *J Neuroimmunol 189*, 59-68.

Wuerbel, H. (2000). *Nat Genet 26*, 263.

VIII-2b-288

The scientific and welfare benefits of the right environmental enrichment for laboratory mice

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Environmental enrichment is most impactful on the wellbeing of an animal when it is biologically relevant, meaning that the item or substrate addresses some behavioral need. Enrichments are especially beneficial if they allow control over the environment or a stressor. One of the few enrichments that meet these criteria for mice is nesting material. Mice under normal laboratory temperatures are thermally stressed, which can compromise aspects of physiology from metabolism to behavior. Over a series of experiments with 3 strains of mice, a nesting material was validated in terms of reducing a physiological stressor with natural coping behavior, improving welfare, and demonstrating end user benefits. Under recommended temperatures all mice preferred 6-10g of nesting material. When provided with 8g, nesting material was shown to reduce radiative heat loss, food consumption, non-shivering thermogenesis, pup mortality, and increase reproduction. One drawback to this amount of material is that mice build fully enclosed nests which prevent observations by animal care staff. Although the mice are not directly visible, nesting behavior shows promise as the basis of an assessment tool for recognizing pain, illness, and distress. An overall reduction in stress, by creating a unique microenvironment, can improve laboratory mouse welfare and scientific outcomes.

VIII-2b-475 *

The Mouse Grimace Scale (MGS): a clinically useful tool?

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Medical research has a heavy and continuing demand for rodent models. Behavioural assessment of pain in such models can be highly time consuming (Roughan, 2003; Miller, 2012) thus limiting the number of models and analgesics that can be studied. Facial expressions are widely used to assess pain in infants, and recently the mouse grimace scale (MGS) has been developed (Langford, 2010). The MGS has shown to be a coding system with high accuracy, repeatability and reliability requiring only a short amount of training for the coder (Langford, 2010). This system therefore has the potential to become a highly useful tool both in pain research and in the clinical assessment of mouse pain.

To date, the MGS has only been used as a research tool; however there is increasing interest in its use in cage-side clinical assessment. Here, we aim to assess the variability in baseline MGS scores between cohorts, sexes and strains. Establishing the presence of a consistent baseline MGS score could lead to a valuable clinical pain assessment tool for mice when prior (baseline) information from the individual mouse may not be available as a comparator. Additionally, the effects of isoflurane anaesthesia on MGS scores will be assessed.

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References

Langford, D., Bailey, A., Chanda, M. et al. (2010). *Nat Methods 7*, 447-449.

Miller, A., Wright-Williams, S., Flecknell, P. and Roughan, J. (2012). *Lab Anim 46*, 304-310.

Roughan, J. V. and Flecknell, P. A. (2003). *Eur J Pain 7*, 397-406.

VIII-2b-684

A global pharmaceutical company initiative: an evidence-based approach to define the upper limit of body weight loss in short term toxicity studies

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This abstract is submitted on behalf of the NC3Rs Acute Toxicity Working Group. Short term toxicity studies are conducted in animals to provide information on major adverse effects typically at the maximum tolerated dose (MTD). Such studies are important from a scientific and ethical perspective as they are used to make decisions on progression of potential candidate drugs, and to set dose levels for subsequent regulatory studies. The MTD is usually determined by parameters such as clinical signs, reductions in body weight and food consumption. However, these assessments are often subjective and there are no published criteria to guide the selection of an appropriate MTD. Even where an objective measurement exists, such as body weight loss (BWL), there is no agreement on what level constitutes an MTD. A global initiative including 15 companies, led by the NC3Rs, has shared data on BWL in toxicity studies to assess the impact on the animal and the study outcome. Information on 151 studies has been used to develop an alert/warning system for BWL in short term toxicity studies. The data analysis supports BWL limits for short term dosing (up to 7 days) of 10% for rat and dog and 6% for non-human primates (Chapman et al., 2013).

Reference

Chapman, K., Sewell, F., Allais, L. et al. (2013). *Regul Toxicol Pharmacol 67*, 27-38.

Session VIII-2: Poster presentations

VIII-2-012

Microenvironment in reusable and disposable individually ventilated cages: a comparative study

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The aim of this study was to compare 3 IVC cages (two disposable, one reusable) in terms of microenvironment quality (intra-cage NH3 gas removal, temperature, relative humidity), bedding weight at cage change and in relation to early indicators of poor welfare in healthy mice: body weight, food and water intake (Burman et al., 2014; Vogelweid et al., 2011; Silverman et al., 2008).

30 C57BI/6 and CD1 male mice, 5 week old male mice were housed in groups of five in Innocage IVC Mouse (disposable Universal Euro II Type Long), SUMC (Single Use Mouse Cage) and GM500 (reusable polysulphone Euro II Type Long) for six weeks. Intracage ammonia level, temperature and relative humidity were recorded every two weeks for three times. Mice and bedding at cage change were also weighed every two weeks, moreover food and water intakes were calculated weekly. Descriptive statistics and one-way ANOVA showed different performances of the 3 IVC systems in the maintenance of the microenvironment quality with the worst ammonia level in Innocage system. Bedding weight increased after two weeks in all cages, but the weight of bedding collected from Innocage system was steadily the highest. Water and food intake as well as mice growth curves were comparable and consistent with the strain features.

References

Burman, O., Buccarello, L., Redaelli, V. et al. (2014). *Physiol Behav 124*, 92-99.

Silverman, J., Bays, D. W., Cooper, S. F. et al. (2008). *JAALAS 47*, 57-62.

Vogelweid, C. M., Zapien, K. A., Honigford, M. et al. (2011). *JAALAS 50*, 868-878.

VIII-2-034

Husbandry and enrichment for the recently protected common European cuttlefish (*Sepia officinalis*)

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Since 1/1/13 all cephalopods have been protected under European Directive 2010/63/EU in research, following Australia, Canada and New Zealand. There is little data regarding their welfare in captivity or standardised best practices (Smith et al., 2013). *Sepia officinalis* is the most used cephalopod in European research, large numbers are kept in public aquaria/zoos and they are increasingly being used in global mari/aquaculture (Correia et al., 2005).

Cuttlefish are prolific inkers and can easily damage themselves when threatened by jetting into the sides of aquaria, these injuries rarely heal. Enrichment can reduce some forms of damaging behaviour but may have negative effects on water quality. We performed welfare assessment experiments, investigating which environments reduce stress indicators, whether the colour of equipment/clothing has an effect on their behaviour, what types of enrichment they used, if facsimiles of their natural habitat would be accepted and various aspects of group living.

We found certain practices significantly reduce the number of stress responses, along with inking and other damaging behaviours, also, welfare can be increased by enrichment that will not detrimentally affect water quality. This is the first study of its kind regarding welfare for these protected, complex and intelligent animals and we provide numerous evidence based recommendations for their welfare in captivity.

References

Correia, M., Domingues, P., Sykes, A. and Andrade, J. (2005). *Aquaculture 245*, 163-173.

Smith, J., Andrews, P., Hawkins, P. et al. (2013). *Journal of Experimental Marine Biology and Ecology 447*, 31-45.

VIII-2-045

Refinement and efficiency through serial microsampling – cost/benefit analysis

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Advances in bioanalytical methods allow for accurate drug level measurement in small sample volumes. Decreased volumes enable serial blood sample collection from one rodent, rather than terminal or sparse sampling from multiple rodents. Decreased variability in data from serial microsampling would be expected with a discrete pharmacokinetic profile from individual animals rather than the composite profile generated from multiple animals. While the reduction in animal use was a clear benefit, the technical challenges associated with the shift from terminal or sparse sampling remained an obstacle preventing broad adoption of the refinement. A cost/benefit analysis was conducted to assess costs, animal use, and data output from terminal, sparse and serial sampling. While sparse sampling results in decreased external costs, internal animal care costs and animal use, technician costs are increased and data output is decreased by half. Serial sampling results in decreased external costs, internal animal care costs, animal use, and technician costs with a minimal decrease in data output. In addition to annual reduction of costs by approximately $200,000 in mouse use by 90%, corresponding reduction of compound requirement and other unquantifiable savings were considered. Based on these findings, management supported broad adoption of microsampling as a standard for all mouse pharmacokinetic studies.

VIII-2-071

Vaccination in lambs – an animal welfare issue?

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Veterinary vaccines are important to improve animal health (Morton, 2007; Roth, 2011). In addition, vaccination is an easy and effective way to reduce pain or suffering and decreases the use of antibiotics (Singer et al., 2003). But, there are also reports about adverse effects. Local reactions at the injection site are a potential animal welfare issue, especially in sheep. The current concepts for clinical studies to evaluate side effects need a considerable number of animals due to pathological examinations performed at different points of time after vaccination. This study tested magnetic resonance imaging (MRI) as a non-invasive tool to evaluate local reactions after vaccination in live lamb. Totally, 32 Merino lambs were vaccinated and scanned at day 1, 3, 8, 15, 22 and 29 after vaccination. Extensive inflammatory reactions could be identified (inflammatory volumes up to 35 cm^3). 27 lambs developed abscesses at the injection site. 50% of these animals underwent a pathologic examination, which reappraised the MRI results. It became apparent that vaccination with licensed products –even under experimental conditions- may cause severe local reactions. MRI seems to be suitable to investigate injection site reactions in live lambs. Using MRI for safety testing may help to avoid such welfare issues in practice.

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References

Morton, D. B. (2007). *Revue scientifique et technique (International Office of Epizootics)* 26, 157-163.

Roth, J. A. (2011). *Procedia in Vaccinology 5*, 127-136.

Singer, R. S., Finch, R., Wegener, H. C. et al. (2003). *The Lancet Infectious Diseases 3*, 47-51.

VIII-2-105 *

Refining oral gavage: assessing and improving welfare in the laboratory-housed dog using a Welfare Assessment Framework

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The dog is a frequently-used, non-rodent species in the safety assessment of new medicines yet little empirical research exists on the assessment of dog welfare and how it is affected by regulated scientific procedures (Prescott et al., 2004). A Welfare Assessment Framework (Hall et al., in prep.) was developed and a contrasting pattern of behaviour, cardiovascular, nociceptive and affective measures was found in dogs with differing welfare states. We present the Framework and an example of its application in Refining a common regulated procedure, oral gavage. Using the Framework, we compared welfare measures across three conditions: Sham Dosing (SD), Refined Training Protocol (RTP) and a Control group that had neither training nor sham dosing, to determine the benefit to welfare and scientific output of each technique. The pattern of findings showed that SD is ineffective as a habituation technique and "primes" rather than desensitises dogs for dosing. Dogs in the Control group showed few changes in parameters across the duration of the study with some indicators of negative welfare during dosing, while dogs in the RTP condition showed improvements in many parameters across the study. We conclude that a training protocol should be implemented for dogs undergoing regulated procedures, while SD protocols should not be used.

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References

Hall, L. E., Robinson, S., Buchanan-Smith, H. M., *in prep*.

Prescott, M. J., Morton, D. B., Anderson, D. et al. (2004). *Lab Anim 38, Suppl 1*, 1-94.

VIII-2-179

Refinement in pain relief in sheep fetal surgery

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Pregnant sheep are commonly used as a model to study placental and fetal hemodynamics. Such studies require major surgeries and adequate pain relief. Although there is some evidence that fetuses do not feel pain, pain alleviation during and after surgical procedures is critical. The aim of this study was to determine fentanyl concentrations in plasma of pregnant sheep and fetuses, in order to refine the pain alleviation program in sheep surgery. Blood samples were taken from animals that were part of an experiment aiming to improve the outcome of the newborns suffering from placental insufficiency. Altogether 21 Aland landrace sheep and one fetus from each sheep were sampled. Fentanyl was administered intravenously to the ewes during the operation. Transdermal fentanyl administration was started perioperatively and continued postoperatively. Maternal and fetal blood samples were collected simultaneously during surgery and fentanyl concentrations were determined with LC-MS. The intraoperative fetal fentanyl concentrations ranged between 0.3 and 0.6 ng/ml, a level considered to be analgesic (0.2-2.0 ng/ml). The fetal/maternal ratio varied between 0.41 and 0.79. However, the assessment of the pain relief must be based on the responses of the fetus and the ewe during the operation and behavior of the ewe postoperatively.

VIII-2-202

Putting animal welfare principles and 3Rs into action

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Promoting good science and animal welfare, and increasing understanding of how the two are intertwined, is an essential enabler of high quality research and 3Rs achievements. The progress we make in this area is one step in enhancing benefits for patients. This poster presents a non-exhaustive inventory of examples from the pharmaceutical industry and its collaborations (http://www.efpia.eu/topics/innovation/animal-welfare):

– Beyond policy principles. Since the late 80's, the European Federation of Pharmaceutical Industries and Associations has an expert group fostering exchange of information and good practice within and across sectors, and promoting development and uptake of 3Rs approaches;
– Many 3Rs methods are a result of joining forces and sharing information. Examples include dried blood spot for toxicokinetic studies, refinement of short term toxicity studies based on body weight loss, and education tools on training animals;
– Beyond compliance. Pharmaceutical companies strive to improve research practice beyond legal requirements through monitoring and auditing research, review and best practice sharing, training on requirements of lab animal legislation;
– Continuous research efforts. Whether aimed at more predictive science or at 3Rs and welfare (IMI – Innovative Medicines Initiative, http://www.imi.europa.eu);

As research paradigms evolve and industry continues its efforts, more dramatic improvements can be expected in the future.

VIII-2-304

Severity classification in German animal research applications

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Directive 2010/63/EU (1) aims to enhance transparency in animal research and requires researchers to classify the severity of procedures prospectively in research applications (Recital 22). This requirement was present in Germany prior to introduction of the revised Directive. For the first time access was granted to over 500 animal research proposals that were authorized in Germany in 2010. These involved procedures in which mice and rats underwent recovery surgery. They were examined to determine how researchers estimated the severity of procedures. Their judgements were compared to the guidance given in Annex VIII of the Directive (2010/63/EU) and to the recommendations of the Expert Working Group on a Severity Assessment Framework (NCA, 2012; EC, 2013). The researchers' use of Refinement was also taken into account in the severity assessment.

The majority of the researchers (63%) underestimated the severity of their procedures. Estimated severity was also often higher than necessary since possible Refinement methods were not always applied. The use of analgesics for example was not routine – 19% of animals that underwent severe procedures did not receive any postoperative analgesia. This presentation will include a range of examples of severity classification and will discuss potential ways to reduce the severity and improve Refinement.

References

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

EC (2013). European Commission Expert Working Group. http://ec.europa.eu/environment/chemicals/lab_animals/pdf/examples.pdf

NCA (2012). National Competent Authorities for the implementation of Directive 2010/63/EU. Brussels. http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Consensus%20doc%20on%20severity%20assessment.pdf

VIII-2-305

Refinement in German animal research applications

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The German Animal Welfare Act (TierSchG, 2006) and Directive 2010/63/EU require researchers to take all practicable steps to minimise pain, suffering and distress (Article 4 (3)). For the first time ever access was granted to animal research proposals that were submitted all over Germany in 2010. They were assessed to determine how effectively these legal obligations were being met. In over 500 research applications involving procedures in which mice and rats underwent recovery surgery Refinement methods, particularly the use of intra- and postoperative analgesia, humane endpoints and health score sheets as well as the monitoring frequency of the animals' health status were evaluated.

This detailed survey indicated that approximately 19% of the animals who underwent severe procedures did not receive any postoperative analgesia. In 57% of the research applications the researcher did not mention any humane endpoints. When considering the severity of procedures the frequency of monitoring the laboratory rodents' well-being should have been higher in most cases. Score sheets were rarely used (13% of applications).

References

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

Tierschutzgesetz (TierSchG) vom 8. Mai 2006, zuletzt geändert durch Gesetz vom 4. Juli 2013 (BGBl I Nr. 36 vom 12. Juli 2013, S. 2182).

VIII-2-311

India frames guidelines for re-use and rehabilitation of dogs and non- human primates in research and testing

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India has boldly come forward to frame necessary guidelines which limit the use of dogs and non-human primates in testing and research. The CPCSEA, the statutory body of the Government of India, which regulates use of animals in experimentation, has formulated guidelines that define a time limit for which dogs and primates can be tested and/or housed in laboratories. The concept of Rehabilitation, has been recognised in India as the 4th R as early as in 2001 (Pereira et al. 2004; Pereira and Tettamanti, 2005), and evolved as an official policy of the CPCSEA in 2004 (Anon.). The guidelines are based on the premise that animals in laboratories undergo psychological, physiological and physical trauma, not just from the interventions made on them, but also from solitary confinement, lack of natural conditions, caging and absence of appropriate social interaction. The guidelines define "re-use" and "rehabilitation" and have limited use of dogs/primates up to a maximum period of 3 years. Repeated use of dogs/primates in regulated procedures is allowed only after liver and kidney function tests confirm that the animal is normal (Fentener van Vlissingen et al., 1997). The guidelines cover the use of dogs/primates in PK studies, telemetry studies, high pain/distress studies, basic bio-medical research and breeding.

References

Anon (2000). LASA Guidance on the Rehoming of laboratory Dogs. In: *A report based on a LASA working party and LASA meeting on rehoming laboratory animals*. Tamworth, UK: LASA,

Anon (2004). Report of the consultative group on review of the norms and practices for regulation of animal experimentation. Ministry of Environment & Forests (Animal Welfare Division) Government of India.

Fentener van Vlissingen, J. M., de Greeve, P. and Verhoog, H. (1997). In L. F. M. van Zutphen and M. Balls (eds.), *Proceedings of the 2nd World Congress on Alternatives and Animal Use in the Life Sciences*, Utrecht, The Netherlands (263-267). Amsterdam, The Netherlands: Elsevier Science B.V.

Pereira, S., Veeraraghavan, P., Ghosh, S. and Gandhi, M. (2004). *Alt Lab Anim 32, Suppl 1*, 411-415.

Pereira, S. and Tettamanti, M. (2005). *ALTEX 22*, 3-6.

VIII-2-327

Determination of the optimal dose of benzocaine hydrochloride in euthanasia of frogs (*Rana catesbeiana*)

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A new guideline concerning laboratory animal euthanasia has been created recently in Brazil. Concerning frogs we had been used a physical method and nowadays only chemical methods with benzocaine or tricaine have been approved and the optimal dosage and the time need to be stablished to induce the euthanasia according to our needs. The aim was to study the best optimal dose of benzocaine hydrochloride to induce euthanasia in bullfrogs to use this method in our University and offer this information to other institutions. In this study we used 10 frogs and the benzocaine was diluted in 50 ml of ethanol, in concentrations of 100, 200, and 300 mg/l of water. The loss of all reflexes and the time of induction was registered. The results were 34 minutes in concentrations of 100 mg/l, 22 minutes with 200 mg/l and 18 minutes with 300 mg/l to induce the euthanasia. These preliminary results demonstrated that euthanasia can be induced using 300 mg/l in 18 minutes. Now we will continue our study increasing the dose to try to decrease the time of induction and compare with tricaine to prove that benzocaine is as good as tricaine and the best due to costs.

VIII-2-372

Cognition studies refined with pet animals

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The study of cognitive processes is an expanding field due to increasing number of mental disorders affecting people. Often these studies exploit laboratory animals and are hard to replace. Pet animals offer an interesting source of information as they share the emotional environment with people. The aim of our study was to increase understanding of attentional cognitive processes common to humans and dogs.

We recruited dog owners and trained them to use positive reinforcements to train their dogs to perform the task necessary for contact free eye movement monitoring. The task was to choose to stay still while shown pictures.

We have used about 50 dogs with only one unable to perform the task. 100% of recruited dog owners volunteered for training and 91% of the volunteers got eventually tested.

Non-invasive gaze pattern recording on pet dogs proved to be successful for modeling shared mammalian cognition. The method allowed better species specific housing conditions (homes) and no inconvenience applied upon animals while volunteering (non-invasive methods, positive training) compared with experiments conducted on laboratory animals (Somppi et al., 2012).

Reference

Somppi, S., Törnqvist, H., Hänninen, L. et al. (2012). *Animal Cognition 15*, 163-174.

VIII-2-379 *

Assessing physiological and behavioral responses over time in laboratory beagles

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To assess adaptation to their laboratory environment, twelve young, naive beagles (6M+6F) and 14 (7M+7F) older, more experimentally experienced Marshall beagles were observed using a standardized procedure: 1) dogs were placed on an exam table and fitted with a

non-invasive Polar® heart-rate monitor; followed by 2) a 5-min observation with dogs standing/sitting on the table lightly restrained by a technician; 10 min after the procedure, 3) a saliva sample was collected for cortisol determination.

Beagles showed variable behavioral responses like licking lips, paw lifting, tail between legs and sniffing table. "Experienced beagles", showed more tail between legs during the 5-min observation ($p<0.05$). We found a large individual variation in heart rate variability and in both morning and post-observation salivary cortisol values, but no significant associations with age, gender or experience.

The beagles' heart-rate responses over time showed four distinct patterns: 1) a high heart rate slightly decreasing; 2) a steeply decreasing heart rate; 3) a fairly constant heart rate; 4) a low heart rate slightly increasing. Further research is needed to verify whether these responses are indicative of the individuals' adaptive capacity in those circumstances, and if they may be useful indicators for estimating potential welfare risk in laboratory beagles.

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VIII-2-460

Refinement strategies to improve the survival rate in mouse models for influenza A virus infections

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Mouse adapted influenza strains have been used to increase the susceptibility to Influenza A (IA) viruses in this model. Mice infected with these viruses could show severe clinical signs, including ruffled coat, depression, anorexia, dehydration, hypothermia, body weight loss exceeding 20% and eventually death. The aim of this study was to investigate possible refinement strategies to improve the survival rate of mice infected with a mouse adapted IA virus. From day 5 to 15 post-infection animals were monitored twice a day and treated with the increase of room temperature at 25°C, and the rehydration with 1 ml of physiological saline (38°C) subcutaneously when 15% body weight loss was reached. However, mortality and survival rate did not show any significant improvement in comparison with previous routine management. Moreover, we proposed the administration of a hydro-gel or corn mush to stimulate food intake, and the early administration of higher volumes of rehydration solution at least twice a day, when animals showed a 10% body weight loss. A better survival rate leading to a reduction of the number of infected animals lost due to poor general conditions and then of the total number of animals used is expected.

VIII-2-553

Update on best practice approaches to the welfare and husbandry of fish, cephalopods and decapods

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Aquatic species are increasingly used in research and testing, in such diverse areas as toxicity testing, environmental monitoring, comparative medicine and genomics. According to the latest (2011) EU statistics, fish species account for 12% of the 11.5 million animals used in research and their numbers have increased by nearly 30% since 2008. The new EU Directive 2010/63/EU now covers cephalopods, and several countries regulate the use of decapod crustaceans in their domestic legislation.

Most guidelines on animal care and use are, however, heavily biased towards terrestrial vertebrates and are often of little value when addressing the welfare and husbandry of aquatic species. The paucity of specific guidelines for aquatic animals reflects the general lack of scientific knowledge of their welfare and husbandry needs, complicated by the large number of species involved (e.g., guidelines for "fish" would not be feasible as there are over 30,000 species).

This presentation will describe current legislation regulating the care and use of laboratory animals in Europe as it applies to fish, cephalopods and decapods. It will also present ongoing work to supplement current legislation with good practice guidelines on welfare, refinement and husbandry. The role of non-governmental organisations in this process will also be discussed.

VIII-2-562

Use of *Eclipta prostrata* extract prevents tissue damage induced by snake venoms immunization in horses during antiofidic serum production

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Introduction: Treatment of snake venomous accidents is a medical and veterinarian challenge. Venoms from these snakes induce edema, hemorrhage and necrosis. Serum obtained from hyperimmune horse plasma is the main treatment of the snake bites. The serum production involves the subcutaneous injection of snake venoms in horses. Although these injections do not cause any systemic repercussions, it promptly causes local edema and tissue damage and in some cases abscess, compromising the animal health. The reduction of these local lesions, without changing the immune response of these animal to the venoms components is a challenge for the serum production centers.
Objective: Add the *Eclipta prostrata* (EP) crude extract to the snake venoms on the horse immunization process at Vital Brazil farm with the goal of reducing the local tissue damage.
Methods: The EP was added to the venoms and injected subcutaneously in horses.
Discussion: The EP reduced in 50% the edema and 100% the abscess formation rate caused by the immunization process in horses compared to the control group.

Our results show that the EP are able to reduce the local tissue damage caused by snake venoms in the immunization process improving the animal welfare and the serum production.

VIII-2-579

Decreased use of experimental animals through optimization of experimental design for efficacy studies

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The aim of this study was to explore whether asymmetric experimental designs can reduce animal numbers without affecting statistical power of the study.

Data from several lung fibrosis studies in mice were combined to estimate expected outcomes for newly planned studies. Classically, these studies comprise a PBS control group, a bleomycin-induced (bleo) group, and several treatment groups with equal animal numbers. Instead of comparing all groups to each other, comparisons of interest were defined in advance: bleo versus PBS control, and bleo versus k treatments (in total k + 1 comparisons). Since the bleo group appears in all comparisons, changing the size of this group will have most effect on the statistical power. The optimal asymmetric design achieves the desired statistical power for each planned comparison with the minimum of animals needed.

We calculated that the best asymmetric designs required about 20-25% less animals than the best symmetric design. This demonstrates that substantial reduction of animal use is possible by smarter choices on group size without loss of power. To aid researchers to optimize their experimental design, we developed a freely available sample size calculator (available at http://www.tno.nl/3R) to compare symmetric and asymmetric designs.

VIII-2-733

Animal welfare issues in air transportation and security services

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Pet animals are often transported by domestic and international flights as per IATA regulation. Major constraints of pet animal transport via air are: 1) Sudden exposure to entirely different climatic condition makes them stressed. 2) Confinement of animals to the new specified and strange container makes them aggressive and off feed creating starvation and dehydration for more than a day. 3) During air transit they get exposed to different atmospheric pressure gradient. 4) Darkness air cargo may make them stressful. 5) On long transport, the container may not be cleaned properly and animal is forced to stay in unhygienic environment. All together air travel can be stressful to dogs and cats. However, proper planning and care before transit, acclimatizing them to the specified crate, feeding on high energy, high fat food before travel, health check-up by a veterinarian and administration of immunity boosters make their travel more comfortable. Dogs are often used by customs and other security agencies to detect narcotics, explosives and other smuggled goods. Their workload and solitary lodging are some of the important welfare issues. Policies and regulations are to be formulated for the welfare of animals engaged in the above services also.

VIII-2-739

Stress response to blood sampling from dorsal pedal vein compared to saphenous vein in mice

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Blood sampling in rodents has historically focused on collecting sufficient blood in a simple, efficient manner. Today, with the development of laboratory tests that can be performed on ever decreasing amounts of serum, or even on a single dried blood spot, one has the opportunity to focus on the welfare aspects of blood sampling. This study used blood glucose as an indicator of stress and found no difference in stress response if the samples were acquired rapidly. However, if the animal was restrained for a prolonged time, blood glucose values increased if collected by saphenous venepuncture. Pedal venepuncture has several advantages over saphenous venepuncture with regards animal welfare and should be the method of choice when small or moderate volumes of blood are to be collected.

VIII-2-812

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

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Micro-isolation 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.

This procedure relies upon the transmission 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

Recent advances in technologies and platforms have enabled other approaches centered on the use of PCR to be proposed, enabling the use of immunodeficient sentinels or relying on environmental monitoring only.

In addition to the improvements in the area of molecular diagnostic, it's now possible to perform the most advanced serological tests in rodents with the use of the dried blood spot technology. This application in conjunction with other laboratory techniques makes non terminal sampling screening of animals possible.

This can be achieved by collecting a single drop of whole blood for serology, faeces and swabs for detection of bacteria and parasites, resulting in a really easy collection procedure, enhancing the 3Rs and possibly, reducing the overall costs of health monitoring programs.

VIII-2-851

Laboratory mouse euthanasia: aversion and refinement

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The most common agents used for laboratory mouse euthanasia are isoflurane and carbon dioxide (CO_2). In Experiment 1, a light-aversion test was used to examine mouse aversion to a rising concentration of CO_2 at a 20% flow rate, 5% isoflurane administered using a vaporizer, or 5% isoflurane administered using the drop method. Mice chose to remain in the chamber longer, and were more likely to become recumbent, when exposed to the isoflurane vaporizer treatment, compared to the other treatments. These results indicate that isoflurane delivered by a vaporizer is a humane refinement for mouse euthanasia. Once mice have been rendered insensible using isoflurane, users may switch to a high flow rate of CO_2 to decrease time to death, but no recommendations exist for when it is safe to switch to potentially painful CO_2 concentrations. Experiment 2 examined three measures of insensibility (recumbency, loss of the righting reflex, loss of the pedal withdrawal reflex) in mice. The results suggest that users should wait a minimum (mean + 3 S.D.) of 79 s after the appearance of recumbency before switching to a high flow rate of CO_2, when using the isoflurane vaporizer method of euthanasia.

Session VIII-3: Humane principles in experimental techniques and benefits of 3Rs

Co-chairs

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Session VIII-3: Oral presentations

VIII-3-062

Applying the concept of wellbeing to the advancement of Refinement

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Although, to date, strategies to identify and alleviate the experience of pain or distress have been the primary focus to achieving Refinement, it is noteworthy that Russell and Burch (Russell and Burch, 1959) contended that we should "aim at wellbeing rather than a mere absence of distress". Further, they concluded that the "psychosomatics of experimental animals" was probably the single most important area by which Refinement would be advanced. This at a time when the mind<>body relationship was contentious, poorly understood and generally not recognised as significant in non-human animals.

The notion of wellbeing implies positive mental state, positive experiences, successful biological function and a capacity to respond to and cope with potentially adverse conditions Anon, 2013).

Recent advances in neurobiology and ethology have provided evidence that animals experience emotional states. Whilst there is increasing evidence of the impact of negative emotional experiences on the validity and interpretation of data, the role and significance of positive experiences merits investigation.

This paper will argue that the concept of wellbeing is pivotal to achieving the goals of Refinement and that a focus on ways to enable cognitive and emotional development will support animal wellbeing, advance the goals of Refinement and potentially enhance scientific outcomes.

References

Anon (2013). Australian Code for the Care and Use of Animals for Scientific Purposes. Canberra: Commonwealth of Australia.

Russell, W. M. S. and Burch, R. L. (1959). London: Methuen & Co Ltd.

VIII-3-415

Fluorescent target array reduces mouse numbers while assessing multiple post-vaccination T-cell responses

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The high-throughput multi-parameter technique, fluorescent target array (FTA) assay, allows the simultaneous quantitation of CTL-mediated target cell killing, functional avidity and epitope variant cross-reactivity in real time *in vivo*. Traditionally these techniques require large numbers of mice for two point assays, peptide titration to optimise CTL avidity conditions, and epitope variant responses. Through the capacity of FTA to measure several T-cell responses simultaneously, via novel multiple cellular staining techniques, many fewer mice were required for the experimental characterisation of cellular responses post anti-viral vaccination (reduction of 6068 to 44 mice). Proof-of-concept was demonstrated via a vaccination protocol that used a recombinant (HIV-1 epitope) fowlpox/vaccinia virus prime-boost regimen in mice, followed by FTA investigations of the cytotoxic $CD8^+$ T-cells (CTL) responses to HIV-1 expression post-vaccination. This study applied the FTA assay as a screening tool to assess over 20 different HIV-1 poxvirus vaccination strategies in mice, and revealed heterologous poxvirus prime-boost vaccination regimes as the most effective for generating high quality CTL responses. The FTA assay revealed important insights into CTL function against HIV-1 infection, while reducing the required number of animals by >100-fold.

VIII-3-583

Microsampling a bioanalytical view

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The last few years has seen the interest in Microsampling dramatically increase, focused initially on the use of dried blood spots. The aim is to use smaller samples for toxicokinetic and pharmacokinetic analysis to reduce the number of animals used on studies and also to refine the data generated. The refinement in the data is to give full profiles from single animals and also to generate TK data from the main study animals allowing correlation of the toxicology data with the exposure data. While interest in microsampling has grown, there has not been a significant increase in its use in regulated studies and therefore the reduction and refinements have not been realised. In this presentation we discuss the perceived and real hurdles to the implementation of microsampling in regulated studies.

Finally, Charles River has implemented microsampling globally across the company and the data from this will be presented for both small and large molecule case studies.

VIII-3-618

Validation of pain-related grimace scales in preweaned laboratory animals

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Despite advances in pain recognition in adult animals, few methods have been validated to assess pain in preweaned laboratory animals, including mouse pups and baby pigs. It is important to have reliable and clinically useful methods for pain assessment since many potentially painful procedures are performed without analgesia on animals of this age, such as tail tipping for genotyping and ear notching for identification in mouse pups, and castration and tail docking of piglets. The objectives of this work were to develop, evaluate, and validate grimace scales and behavioural scoring as tools to assess pain in preweaned mice and pigs, and to use these tools to assess efficacy of analgesic agents for addressing potentially painful conditions in mouse pups (ear notching; carprofen) and boar piglets (castration; prilocaine/lidocaine cream and/or meloxicam). In studies with both species, facial action units were identified and then scored by individuals blinded as to animal treatment. For each species, comparing the change in facial action unit scores from baseline to post-procedure with changes in baseline behavioural scores with and without analgesia was used to validate grimace scale scores. Close correlation was demonstrated, suggesting that grimace scales have clinical utility for pain assessment in preweaned animals.

VIII-3-775

Decreased levels of discomfort in trained mice at experimental procedures, assessed by facial expression

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A need for more objective approaches on measurements and assessments of improved welfare has been identified. In this study, mice were trained to acclimatize to a new method for non-restrained subcutaneous injections. The test group (n=20) was trained five days per week, while the control group (n=20) was handled without training once weekly. After three weeks, both groups were injected subcutaneously. A modified protocol for assessment of facial expressions of pain in mice (Langford et al., 2010) was used, with ear and eye scoring at a three grade scale: 0=normal, 1=slightly, and 2= totally changed/altered. Six blinded experienced animal technicians scored the test group days 1 and 7, and both groups day 22. After one week of training, ear and eye scores significantly decreased (Ears: from 1.7 to 1.3; Eyes from 0.9 to 0.6). In addition, trained mice displayed significantly lower ear scores during subcutaneous injections than controls (0.75 versus 1.1). These data clearly show that trained mice display less discomfort than non-trained mice during subcutaneous injections, assessed by ear scoring, and that preparing animals to experimental procedures has a 3R potential. Facial expression scoring can be an important tool when assessing improvements of animal welfare in 3R projects and method development.

Reference

Langford, D. J., Bailey, A. L., Chanda, M. L. et al. (2010). *Nat Methods 7*, 447-449.

VIII-3-853

European refinement initiative – science-based refinement

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During the workshop "Science-based Refinement" hosted by CAAT-Europe, Novartis International, Hoffmann-LaRoche and BSL Bioservices, experts from industry, academia and animal welfare came together to discuss new approaches in the area of refinement.

Special topics were "Experimental Design" and "Animal Welfare Indicators". As already stated by Russell and Burch (1959), it is always becoming clearer that compromises regarding animal welfare disturb the quality of science (Knight, 2001).

The session "Animal Welfare Indicators" provided a new and more dynamic concept of animal welfare (Ohl and van der Staay, 2012), discussed missing pain assessment and analgesics application in labo-

ratory animals, as well as end point refinement in rodents. Disturbing results on euthanasia were shown and it became clear that "a humane death" in our opinion might not be "humane" for the species, meant to endure the procedure.

"Experimental Design" dealt mainly with publication bias (Sena et al., 2010) and excess significance (Tsilidis et al., 2013), drew groundbreaking conclusions on the quality of animal studies and pleaded for a better generalizability of results. The "collaborative approach to meta analysis and review of animal data from experimental studies" (CAMERADES) presented themselves, their goals and successes.

The summary report with all facts, conclusions and recommendations will be another step towards a science-based refinement in laboratory animal studies.

References

Knight, J. (2001). *Nature 412*, 669.
Ohl, F. and van der Staay, F. J. (2012). *Vet J 192*, 6-7.
Russell, W. M. S. and Burch, R. L. (1959). London: Methuen & Co Ltd.
Sena, E. et al. (2010). *PLoS Biol*.
Tsilidis, K. et al. (2013). *PLoS Biol*.

Session VIII-3: Poster presentations

VIII-3-106

Principles of animal modelling in psychiatric research with respect to 3R rules

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At the moment, with respect to the long-term development of this field, animal models are viewed as highly valuable and extensive tools in biomedical research.

To mimic brain pathogenesis in human neurodevelopmental disorders, many model studies are done using rodents, with whom we can evaluate many patterns in the ethogram exhibited by the species used in specific experimental situations. It has recently become increasingly important to develop animal models that enable multiple behavioural domains to be explored in parallel and thus reduce the number of animals used as much as possible (the rule of reduction). The solution in this instance is to use more behavioural endpoints per experiment, which allows a greater number of different domains to be registered. For example, preclinical studies suggest hyperlocomotion in rodents to be equivalent to positive symptoms of schizophrenia. Moreover, exploration can be used as a marker of vulnerability to stress or anxiety. The rule of refinement is closely connected with correctly respecting the rules in experimental manipulations and standardizing testing conditions in order to attenuate interindividual variations. However, it is very difficult to fully implement replacement in animal models of neuropsychiatric disorders, due to the complexity of brain functions and brain pathogenesis.

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VIII-3-736

Gavage incidents – a urgent need for action

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Gavage is an extensively used technique for the oral dosing of substances. The technique involves the restraint of conscious animals and insertion of a rigid metal or flexible plastic tube into the oesophagus to deliver a bolus of substance directly into the stomach. Research has shown that, even when carried out properly, the procedure can induce significant stress, which could confound research results. Additionally, there is a risk of gavage-incidents, commonly reported as deaths. Gavage incidents include accidental insertion into the trachea causing suffocation, puncturing of the throat or stomach and chronic irritation leading to severe weight loss or excessive ingestion of bedding. Mortalities have ranged from 0 to 53% in single studies but to date the extent of gavage incidents across a sector is not yet known.

The aim of this study was to determine the incidence of gavage-related mortality in regulatory toxicology submissions for industrial chemicals. Over 300 independent studies were reviewed from the ECHA CHEM database for reports of gavage- attributed deaths. We observed an unacceptably high proportion of studies with gavage incidents. More humane approaches to dosing do exist but are under used- perhaps due to a failure to appreciate the extent of gavage related deaths.

VIII-3-923

The development of hormone loaded diets to promote xenograft growth

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In our facility, we use several tumours which are dependent on hormone supplementation, delivered via subcutaneous pellets, for growth. Due to supply issues, we had to develop an alternative method of DHT supplementation and decided to try delivery via a fortified diet, as this would be less invasive and stressful to the animals and more convenient for the staff. After development, the diet was tested against the LNCaP prostate tumour line and was found to be as effective in stimulating growth as pellets. We then decided to adopt the same method for estradiol delivery hoping it would eliminate associated side effects, and MCF-7 breast carcinoma growth was stimulated as required with side effects markedly reduced.

We believe delivering hormones via the diet, rather than pellets, is a major refinement in welfare terms by reducing both the need for invasive implants and in side effects, while still promoting tumour growth.

VIII-3-938

Humane principles of using animals in Russia

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Russia's lack of documents regulating the ethical principles of the treatment of animals is the cause of the difficulties of international relations in various fields of science and animal husbandry. Matter of moral attitudes to animals is relevant throughout the world and characterizes the level of civilization of the country. As a result of literature search we have found that in the history of Russia there were only 5 regulations governing ethics in dealing with animals (Selezneva and Makarova, 2014; Kopaladze, 1998). Animal Protection Act in Russia has not been accepted. In Russia today are no documents to regulate the ethical principles of biomedical research involving animals. Most of the leading research organizations and our organization too, adhere to the principles of 3Rs and Directive 2010/63/EU (Russell and Burch, 1959; Directive 2010/63/EU; http://www.nc3rs.org.uk). The 3Rs are a widely accepted ethical framework for conducting scientific experiments using animals humanely.

The level at which Russia is in this matter, perhaps resulting in the low credibility, both scientific research and the country as a whole. Thus, the creation of the Russian legal framework ethical treatment of animals is necessary in our opinion. And this database must comply with the principles of 3Rs.

References

Directive 2010/63/EU. *Journal of the European Union*, 33-79.

Kopaladze, R. A. (1998). *Usp Fiziol Nauk 29*, 74-92.

Russell, W. M. S. and Burch, R. L. (1959). London: Methuen & Co Ltd.

Selezneva, A. and Makarova, M. (2014). *International Bulletin of Veterinary Medicine 1*, 69-75.

Session VIII-4: Avoidance of severe suffering

Co-chairs
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Session VIII-4: Oral presentations

VIII-4-342

Humane end-points in animal experimentation

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Biomedical research without the need to use laboratory animals is what most of us are aiming for. However, replacement is a long and tedious road and it might be anticipated that we will continue performing animal experiments for the next few decades, including experiments that are known to induce severe pain and distress, e.g., infectious disease models with lethal challenge procedures or tumour survival studies. Particularly the high severity category of experiments requires our special considerations; for animal welfare concerns, but for scientific and legal reasons as well. Our last resort in experiments with significant pain and distress is to limit the time period animals are severely suffering. This approach is generally referred to as applying humane end-points.

In this presentation I will make a distinction between unavoidable and avoidable pain and distress and the consequences for applying humane end-points. I will discuss various types of humane end-points, including opportunities to introducing non-clinical end-points. Finally, following my conclusion that implementation of humane end-points still is far from optimal, I will address existing barriers and imitations to implementing humane end-points as part of a "culture-of-care" attitude in the laboratories.

VIII-4-523

Inflammation imaging and refinement of post-surgical NSAID dose recommendations in mice

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NSAIDS treatments are common welfare interventions with the aim of reducing post-operative pain linked to tissue inflammation. However, dose recommendations derived from indirect assessments (e.g., behaviour, hyperalgesia) may be ineffectual, particularly in mice, where questionably large doses seem to be needed. We attempted a more direct determination of the anti-inflammatory effects of meloxicam (1, 5 or 20 mg/kg s/c) given 1 hour before laparotomy in groups of male BALB/c mice (n=9-12) by imaging a fluorescent COX-2-targeted probe (Uddin et al., 2010) at 7, 24 and 48 hours. Results were contrasted with efficacy/welfare estimates from body weight and automated behaviour analyses (Roughan et al., 2009) and the Mouse Grimace Scale (MGS) (Langford et al., 2010). Meloxicam dose dependently reduced inflammation relative to controls. Surgery predictably caused weight losses and abnormal behaviour changes, and increased MGS scores. Meloxicam had no detectable beneficial effects on welfare at any dose or time-point and 5 and 20 mg/kg further increased MGS scores. Imaging confirmed meloxicam's anti-inflammatory effects, so the lack of positive outcomes suggested pain derived from factors in addition to inflammation, which may be only partially controlled by relatively large NSAID doses (Matsumiya et al., 2012; Wright-Wil-

liams et al., 2007) that with current welfare evaluation methods some strains may appear to respond to poorly (Roughan et al., 2009)

References

Langford, D. J., Bailey, A. L., Chanda, M. L. et al. (2010). *Nat Methods 7*, 447-449.
Matsumiya, L. C., Sorge, R. E., Sotocinal, S. G. et al. (2012). *JAALAS 51*, 42-49.
Roughan, J. V., Wright-Williams, S. L. and Flecknell, P. A. (2009). *Lab Anim 43*, 17-26.
Uddin, M. J., Crews, B. C., Blobaum, A. L. et al. (2010). *Cancer Res 70*, 3618-3627.
Wright-Williams, S. L., Courade, J.-P., Richardson, C. A. et al. (2007). *Pain 130*, 108-118.

VIII-4-580

A refined model of experimental autoimmune encephalomyelitis (EAE) to study neuroprotection in multiple sclerosis (MS)

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Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS). Although recent efforts to control relapsing-remitting MS have led to the successful development of new therapies, a neuroprotective therapy to treat the progressive stages of MS is yet to reach the clinic. Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model to study MS. However, EAE is considered a severe procedure due to the nature of disease induction and development of hindlimb paralysis resulting in severe animal suffering.

To refine and reduce the EAE model, a novel animal model was developed using the visual system as a tool for studying neurodegeneration in the CNS. A C57BL/6 mouse expressing transgenes for a myelin oligodendrocyte glycoprotein-specific T cell receptor was crossed with a C57BL/6 mice expressing cyan fluorescent protein (CFP) under control of a Thy1 promoter restricted to retinal ganglion cells (RGC). The resultant mice develop optic neuritis and RGC loss that can be monitored longitudinally using techniques that correlate with human studies (visually evoked potentials, scanning laser ophthalmoscopy and optical coherence tomography). The novel model avoids severe suffering associated with EAE and offers a rapid screening tool for neuroprotective therapies in MS.

VIII-4-688

A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an endpoint: towards adoption of the Fixed Concentration Procedure

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This abstract is submitted on behalf of the NC3Rs Working Group on the Fixed Concentration Procedure (FCP). Acute inhalation studies are conducted in animals as part of chemical hazard classification. Current accepted methods use death as an endpoint (OECD TG403 and TG436). The FCP (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with "evident toxicity", defined as clear signs of toxicity that predict exposure to the next highest concentration will cause severe toxicity or death in most animals. TG433 was dropped from the OECD work plan in 2007 because of a lack of evidence for comparable performance with TG403 and TG436, suspected sex differences (FCP originally only used females) and the ill-defined and subjective nature of evident toxicity. The first two issues have been resolved (Price et al., 2011; Stallard et al., 2011). A global initiative including 20 organisations, led by the NC3Rs, has addressed the last concern with the aim of making evident toxicity more objective and transferable between laboratories. The group has shared data on clinical signs recorded during acute inhalation studies for 188 substances. Preliminary results suggest signs including bodyweight loss, irregular respiration, gasping, ano-genital staining or hypoactivity are highly predictive of severe toxicity or death at the next highest dose.

References

Price, C., Stallard, N., Creton, S. et al. (2011). *Hum Exp Toxicol 30*, 217-238.
Stallard, N., Price, C., Creton, S. et al. (2011). *Hum Exp Toxicol 30*, 239-249.

Session VIII-4: Poster presentations

VIII-4-234

Promising *in vitro* alternatives for nutrition studies in cattle

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Nutrition studies in cattle require rumen fistulation, a surgical procedure creating pain, trauma, and appalling appearance. Rumen Simulation technique (RUSITEC), *in vitro* gas production techniques (IVGPT) and Tilly and Terry method are alternatives to rumen fistulation. In this study RUSITEC and IVGPT are compared for their efficiency to establish rumen bacteria *Butyrivibrio fibrisolvens*. RUSITEC system is a semicontinous culture system consisting of eight vessels (capacity 800 ml) and IVGPT is a batch system with 35 glass syringes (100 ml capacity). Both the system was supplied with rumen liquor collected from slaughtered cattle and artificial saliva, maintained at 39°C and shaken periodically. A complete feed (roughage concentrate ratio: 65:35) supplemented with 4.5% sunflower oil was the experimental feed. RUSTEC vessels were supplied with 10 g feed and 80 g rumen contents in nylon bags and IVGPT syringes were supplied with 200 mg feed. Anaerobic roll tube technique was used for the culture of *B. fibrisolvens* in the rumen fluid collected at the 48th hour. Colony counts were 1.14 x 10^2 ±3.53 in IVGPT and 3.45 X 10^4 ±1.21in RUSITEC. Therefore, RUSITEC is considered as the best laboratory alternative to avoid painful rumen fistulation.

VIII-4-555

A road map towards ending severe suffering

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Revision of the EU Directive controlling experiments on animals has focussed attention on the need to reduce animal suffering in scientific procedures. Classification of levels of suffering into mild, moderate and severe and the need to report actual levels of severity has provided added impetus to the drive to refine the most severe models and procedures, as has greater recognition that high levels of suffering impact on an animal's physiological responses, increasing variability of experimental data. So ending severe suffering is a desirable goal for scientific as well as moral and legal reasons.

This is therefore an excellent time to look at the sources and nature of suffering within the research context (to perform a "severity audit"), to evaluate the effectiveness of current refinement practices and to seek more effective ways of avoiding or minimising all unnecessary pain and psychological distress experienced by animals. Central to the success of such an initiative is a receptive institutional culture and a robust and challenging ethical review process.

This talk will outline the key questions and practical considerations that establishments need to address in order to reduce suffering for all animals and to work towards ending severe suffering.

Session VIII-5: Culture of care

Co-chairs
Jann Hau, University of Copenhagen, Denmark
Susanna Louhimies, DG ENV, EU Commission, Belgium

Session VIII-5: Oral presentations

VIII-5-137

The impact of AAALAC accreditation on compliance with animal welfare laws

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Accreditation of animal research facilities by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) is widely considered the gold standard of commitment to the well being of nonhuman animals used in research. AAALAC-accredited facilities receive preferential treatment from funding agencies and are viewed favorably by the general public. Thus, it bears investigating how well these facilities comply with animal research regulations. In this study, the incidence of noncompliance with the United States (US) Animal Welfare Act (AWA) at AAALAC-accredited facilities in the US was evaluated and compared to those at non-accredited institutions over a period of two years. Our analysis reveals that AAALAC-accredited facilities are frequently cited for AWA noncompliance items (NCIs). Further, AAALAC-accredited sites had significantly more AWA NCIs on average than non-accredited sites. This gap widens as the number of animals per facility increases. AAALAC-accredited sites also had more NCIs related to improper veterinary care, personnel qualifications, and animal husbandry. This study is the first one to demonstrate that AAALAC accreditation does not improve compliance with regulations governing the treatment of animals in laboratories.

VIII-5-153

Promoting an institutional culture of care and ethics

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AAALAC International has conducted almost 700 on-site assessments of animal care and use programs around the globe since its adoption of the eighth edition of the *Guide for the Care and Use of Laboratory Animals* (NRC 2011) as one of its primary standards for accreditation. In analyzing the findings from these site visits, some patterns of challenges institutions are facing in meeting new recommendations in the Guide have been detected. Data from various regions of the world, with reference to institutional oversight, occupational health and safety, animal environment, the program of veterinary care and physical plant will be discussed to assist institutions in proactively addressing these program areas. The dataset generated from reviewing animal care and use programs around the world is unique and can serve as a valuable resource in promoting research animal welfare and high quality science. This information is described at a level of detail that will allow institutions to assess where program enhancements can be effected on the path of continuing improvement that is the foundation of an institutional culture of care and ethics.

VIII-5-169

Culture of the 3Rs: accelerating the development and the implementation of alternatives

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Biomedical research is one of the undisputed areas where progresses represent rapid and major improvements for human and animal health and well-being. Everyone working in this field is aware that the use of animals for scientific purposes is not the only way to address the challenges. All research programmes to be comprehensive include non-animal models and sometimes clinical trials.

Lack of performance indicators for the 3Rs induces the false idea that the research community neglects the non-animal models. The holistic approach of biomedical research programme demonstrates the commitments towards more predictive and accurate models. In addition, mechanism of action could be better analyzed. Culture of the 3Rs highlights the situation and the relative positioning of animal use within the research programme. Moreover, Culture of the 3Rs, when established as an institutional programme is an accelerator of development and implementation of alternatives. The author will present the programme as established by Sanofi Pasteur globally and the benefits for 3Rs implementation.

VIII-5-254

Toenail trimming in mice – an alternative treatment for ulcerative dermatitis

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In 2010 technicians at our university became concerned about incidents of mice in our facility with ulcerative dermatitis (UD).The affected areas were pruritic and subsequent scratching by the mice, often in an obsessive manner, resulted in sore lesions developing.

Husbandry techniques were adapted and veterinary recommended treatments were administered but little improvement was seen. In 2011 a new approach was adopted, trimming the toenails of affected mice in order to break the itch/scratch cycle, instead of attempting to treat the UD itself. This method reduced the amount of skin damage occurring during scratching and the lesions began to heal. Daily photographs taken for ten days documented the healing process and eventual hair regrowth. Extensive data collection on all mice presented with UD enabled comparable analysis of the toenail trimming method against two other treatments, topical application of an antibiotic/anti-inflammatory gel and an IP injection of a synthetic protein thought to inhibit the itch reflex. Data analysis showed that toenail trimming resulted in much higher rates of healing than the other two treatments. As this method is also free, quick, non-invasive and simple to teach, it has now been adopted as the standard treatment of this condition at our institute.

VIII-5-486

What constitutes a good culture of care?

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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 requires that Animal Welfare Bodies should foster a culture of care, to ensure appropriate animal welfare care and use practices are maintained at all times. However, the responsibility and foundation for a good culture of care goes beyond that of just Animal Welfare Bodies – it rests with everyone dealing with animals bred or used for scientific purposes.

Many factors contribute to the establishment and maintenance of an appropriate culture within an establishment. These include:

- Corporate expectation of high standards with respect to the legal, welfare, Three Rs and ethical aspects of the use and care of animals;
- Demonstrable support from senior management;
- Purpose built institutional framework and appropriate tools;
- Shared responsibility and accountability at all levels for care and use practices within the establishment;
- Empowerment – all voices and concerns heard;
- Attitude – all staff appropriately trained and supported, with the "right attitude";
- Proactive not reactive.

The culture of care is never static – it needs to be continually reviewed and reinvigorated.

VIII-5-663

Achieving a good 'culture of care' – what and how

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Establishing, promoting and maintaining a good "culture – or climate – of care" within individual establishments where animals are used in scientific procedures, is a fundamental requirement if legal, ethical and animal welfare obligations, along with wider responsibilities towards staff and to the public, are to be met.

However, although the term "culture of care" is increasingly used within legislation regulating animal experiments, and in related guidelines and policy or position statements, the nature of such a culture is not defined. Essentially, a wide range of components (including leadership, engagement, empowerment, accountability, communication, knowledge, attitudes, training and respect) have to come together to provide an internal framework which delivers truly high standards of animal welfare and science which go beyond the minimum standards of legislation.

This presentation will suggest a definition of "culture of care" and review its key aspects. It is intended to stimulate thoughts and discussion as to how a true "culture of care" can be established, maintained and assessed, and where the responsibility for achieving such a culture lies.

VIII-5-693

Implementing an action plan for world class animal care and husbandry at Imperial College London

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Following publication in 2013 of an independent review by Professor Steve Brown (Brown, 2013) about how Imperial College London (2014) could deliver change to ensure it was a leader, both within the UK and internationally, in its animal care and husbandry, and in developing and applying the 3Rs, in January 2014 the College published its action plan to achieve this objective.

This presentation, offered as part of the Culture of Care session of Theme VIII, would focus on the practical challenges of improving the culture of animal welfare alongside delivering world research involving animals.

The four main commitments the College has made under its action plan are to strengthen its strategic leadership of this area, to promote fuller consideration of the 3Rs through strong links between researchers and animal faculty staff, to reform ethical review processes and to communicate more effectively both externally and internally.

To be successful in achieving these commitments, Imperial College has sought to engage widely in search of best practice for managing world leading, complex multi-site animal research facilities. A substantial reform of the governance of animal research is being implemented.

References

Brown, S. (2013). Independent Investigation into Animal Research at Imperial College London: http://brownreport.info/wp-content/uploads/2014/02/The-Brown-Report.pdf.

Imperial College London (2014). Action plan for world class animal research: https://workspace.imperial.ac.uk/college/Public/ActionPlanAnimalResearch.pdf.

Session VIII-5: Poster presentation

VIII-5-400

Changing the culture of laboratory rat care

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Standard laboratory rat housing generally consists of a small cage, bedding, and a rudimentary shelter. Rats are sometimes habituated to standard laboratory procedures but are rarely socialized to humans. Because rats are inquisitive by nature and able to experience a range of positive and negative emotions, these standard laboratory conditions are likely to affect their welfare (Makowska and Weary, 2013). We housed rats in standard (six pairs) and semi-naturalistic (six groups of five) cages. When housed in the semi-naturalistic cages, Sprague-Dawley rats purchased from Charles River Canada spontaneously dug burrows, climbed, bounded, and stretched to their full capacity, all behaviours which were never seen and were not possible in the standard cages. In another study, a rat socialization protocol was developed that allowed us to raise rats that were friendly and playful even with strangers. These rats, when called, also willingly ran up to and climbed into a testing apparatus. These examples can inform changes in the way we view and care for rats. Dogs and cats used in research "should be allowed to exercise and provided with positive human interaction" (NRC, 2011); we suggest that this guideline should also be applied to laboratory rats.

References

Makowska, I. J. and Weary, D.M. (2013). *Appl Anim Behav Sci 148*, 1-12.

NRC (2011). *Guide for the care and use of laboratory animals* (58). Washington, DC: The National Academies Press.

Session VIII-6: Transgenic animals – Approaches to reduction and refinement in the production and use of GM mice

Co-chairs
Coenraad Hendriksen, Utrecht University, The Netherlands
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Session VIII-6: Oral presentations

VIII-6-182

Refinements for implant surgery: the effects of different anesthetic agents on pregnancy and pup survival

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The most critical aspect of generating genetically engineered mice is the ability to produce live animals for analysis after the appropriate injection procedure. Animals are produced by implantation of manipulated embryos into pseudo-pregnant females for gestation, parturition, and subsequent growth to the weaning stage. Animal loss can occur during any of these stages, resulting in repeated procedures and increased animal usage. One might predict that the anesthesia used during implant surgery could affect the number of pups produced. Anesthetic agents commonly used in the United States for implant surgery include Avertin (a tri-bromoethanol, tert-amyl alcohol mix) delivered by IP injection, ketamine:xylazine (100 mg/kg: 5 mg/kg) delivered by IP injection, and inhaled isoflurane (2.5% in oxygen). To determine if the type of anesthesia used would affect the number of animals produced, we tested each type in implant surgeries and assessed the numbers of pups produced. Sufficient numbers of embryos and implants were used to ensure an appropriately powered study. The results of this analysis will be presented.

VIII-6-310

INFRAFRONTIER-EMMA's contribution to 3Rs and the significant reduction in the number of experimental animals

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Recent progress in molecular genetics and developmental biology has created thousands of newly engineered mutant strains of laboratory animals, including genetically altered (GA) mice, flies, nematodes and zebrafish. This trend is irreversible and will in fact accelerate its pace following the sequencing and analysis of whole human and mouse genomes (2001 and 2002), as permanent resources for targeted mutagenesis in all protein-coding genes (2012). Worldwide, large-scale archiving and distribution network infrastructures, like INFRAFRONTIER-EMMA, will further increase the need to keep and move mutant strains, and particularly GA mice, around the world. Alongside the advances in molecular biology, mouse stock management and cryopreservation procedures are being continually refined and implemented. Maintaining and disseminating mouse stocks as frozen materials offers valuable ethical and logistical advantages over live animal shipment, minimizing the associated welfare issues. Embryo freezing has traditionally been the method of choice for archiving mouse lines, while sperm/oocyte and ovary freezing are emerging as more convenient alternatives, due to the application of innovative protocols. Thus, INFRAFRONTIER-EMMA's application of state-of-the-art technologies in breeding, archiving and transfer of mouse stocks is an essential tool for the 3Rs mission and vision (Wilkinson et al., 2010; Bradley et al., 2012; Guan et al., 2012).

Proposal PART B FP7 – CAPACITIES. Development of mouse mutant resources for functional analyses of human diseases – Enhancing the translation of research into innovation INFRAFRONTIER-I3 Combination of Collaborative Project and Coordination and Support Action for Integrating Activities

References

Bradley, A., Anastassiadis, K., Ayadi, A. et al. (2012). *Mamm Genome 23*, 580-586.

Guan, M., Marschall, S., Raspa, M. et al. (2012). *Mamm Genome 23*, 572-579.

Wilkinson, P., Sengerova, J., Matteoni, R. et al (2010). *Nucleic Acids Res 38* (Database issue), D570-576.

VIII-6-370

Quantitation of fluorescence intensity in mice – a novel genotyping approach of fluorescently labeled transgenic mouse models

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Fluorescence proteins have been useful as genetic reporters for a wide range of applications in biomedical research. Several fluorescent markers with sufficient brightness and spectral separation are available and frequently used for the analysis of transgene activity.

Here we show that transgenic mice with different coat colours ubiquitously expressing a green (eGFP) or red fluorescence protein

(mCherry or tdTomato) can be reliably genotyped by measurement of the fluorescence intensity. We identified the tail skin of the mouse as the tissue best suited for such an *in vivo* genotyping approach. The fluorescence intensity not only distinguishes wild type from transgenic mice but also allows for the reliable determination of zygosity. The results obtained by quantitation of fluorescence intensity were confirmed by standard PCR analysis or test breeding. This novel approach can be used on juvenile or adult animals and allows for instant genotyping without DNA analysis. The feasibility of genotyping without tissue sampling is an important animal welfare aspect.

In summary, we demonstrate for the first time that analysis of ubiquitously expressed fluorescence proteins in transgenic mice can be reliably used to substitute DNA-based genotyping methods or test breeding.

VIII-6-620

Surplus animals in breeding: is there room for reduction?

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Recently, an international expert workshop was held in the Netherlands, entitled: "Bred but not used". These are the animals that are purposely bred but eventually not used in procedures. In the Netherlands, the numbers of these animals have increased steadily over the past 5 years. In 2012, 579,338 animals were used in procedures while 524,735 were "bred but not used". These include animals not suitable for experimental purposes because of their genetic make up, age and/or sex. The figures have raised concern among the public at large and the Dutch authorities. Therefore, the authorities invited twenty-two international experts on the 3Rs, animal welfare, ethics, colony management, molecular biology and laboratory animal science to discuss trends and possible actions. The following themes were identified: moral and ethical aspects; management and technology; education, training and communication; alternatives. Discussions led to an in depth analysis of these aspects of "bred but not used" and the presentation of recommendations to the authorities. The minister has included some of these recommendations in her Action plan: "Animal testing and alternatives".

VIII-6-943

Generation of genetically modified mouse models: the role of the International Society for Transgenic Technologies in refinement and reduction

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After the development of technologies to efficiently manipulate the murine genome during the 1980s the generation and use of model organisms in many areas of basic and biomedical research is without alternative at present. With the proliferation of technologies, networks have formed that have provided for an exchange among each other. From these networks emerged in 2006 the International Society for Transgenic Technologies (ISTT). The ISTT sees itself as a meeting place for all those who generate and use genetically modified animals – currently still mainly mice and rats. The aim of the society is to disseminate techniques and their improvements and to facilitate the optimal use of these techniques in all laboratories employing those technologies. It has become obvious that optimization of methods leads to a reduction of the number of animals required for the generation of genetically modified animal models. Thus, the Society is a major contribution to the reduction of animal numbers. Moreover, through the dissemination of improved surgical techniques, it also contributes to Refinement. Over the last year to this day the application and optimization of new techniques of nuclease mediated gene modification is being discussed among society members. Again the driving influence of the ISTT to the dissemination of improved methods and the potential to reduce animal numbers by an increased efficiency through the implementation of state of the art technologies becomes apparent.

VIII-6-944

Nuclease technology reduces animals use and improves timeline for making mouse models

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Since the discovery of Zinc Finger Nucleases in gene editing in rat genome in 2009 (Geurts et al., 2009), Nuclease Technology for gene editing has transformed the landscape for creating genetically modified rodent models in facilities that have implemented this method. Additionally, this technology is not limited by the use of embryonic stem cells thus can be used to modify the genomes of any species. Recently, CRISPR/Cas9 system allows creating multiple mutations simultaneously (Wang et al., 2013). This report focuses on the impact of the technology on the number of animals used, and the timeline for generating mutant mouse models. Our data show an 85% reduction of mice used by using Nuclease Technology compared with gene targeting through ES cells. The timeline from project initiation to homozygous mutant mouse models born for single mutation was reduced from 16 months to 2 months. For adding a mutation to a single mutation, the timeline was reduced from 18 to 7 months, and for adding a mutation to a double mutation, the timeline was reduced from 21 to 7 months. The results demonstrate that Nuclease Technology is an effective method for generating mouse models while also significantly reducing the number of animals used.

References

Geurts, A. M., Cost, G. J., Freyvert, Y. et al. (2009). *Science 325*, 433.

Wang, H., Yang, H., Shivalila, C. S. et al. (2013). *Cell 153*, 910-918.

VIII-6-947

Reducing and verifying off-target effects when generating genetically modified animal models using genome editing technologies

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In recent decades, using animals to simulate human diseases has provided a means to study pathologies and explore therapeutic agents. Genetically tractable diseases have mainly been studied using genetically modified mouse models such as knockout, knock-in and transgenic mice. Despite mice being one of the most suitable animal models for studying gene functions and human diseases (Waterston et al., 2002; Collins et al., 2003), several aspects of murine biology limit its utilization and result in inappropriate usage of experimental mice. Lack of embryonic stem (ES) cell culture technologies is one barrier preventing the generation of genetically modified animals other than mice. However, recent advances have provided the possibility of producing genetically modified animals by directly applying zygotes using novel genome editing technologies such as ZFN, TALEN and CRISPR. These technologies provide the possibility of production of more suitable animal models with more precise etiology to study human diseases and reduce the improper utilization of experimental animals. However, it is necessary to take into account that putative off-target effects of genome editing technologies might interfere with interpretations and result in ambiguous conclusions. Therefore, there is an inevitable issue about how to reduce and verify off-target effects in generating genetic modified animal models using genome editing technologies.

References

Collins, F. S., Green, E. D. and Guyer, M. S. (2003). *Nature 422*, 835-847.

Waterston, R. H. et al. (2002). *Nature 420*, 520-562.