Theme VIII – Refinement and Animal Welfare

Coordinators
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Session VIII-1: Oral Presentations

Session VIII-1: Evolution of Research Animal Welfare

Co-Chairs
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VIII-1-548
Between evidence base and speciesism – A brief history of laboratory fish welfare
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Early milestones in the advancement of clinical fish welfare and welfare science include the first use of MS 222 by R. Schöttger at the Wisconsin Sports Fishing Laboratory in the 1960s and the behavioural assays on carp by J. Verheijen in the Netherlands in the 1970s and 80s. The latter remained relatively obscure but re-emerged in the 1990s and 2000s after the publication of studies by L. Sneddon, F. Huntingford, V. Braithwaite and others linking new findings on behavioural ecology of fish with are appraisal of their welfare needs. The resulting shift in the perception of fish sentience has triggered a perennial debate on the capacity of fishes for higher conscience with the detractors mainly quoting an antiquated speciesist paradigm.

Over the last five years new advances have come through on the aversiveness of common fish anaesthetics, on utility of environmental enrichment and, more recently, the use of immersion analgesics in fish procedures. Ongoing studies explore anaesthetic efficacy and new setups incorporating sedative pre-medication. It is hoped that the knowledge gained through the laboratory refinement drive will benefit fish welfare in all areas.

VIII-1-681
Evolution of refinement – From concept to practice
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The Three Rs of Russell and Burch have provided a solid foundation for the current legislative framework for animal use and care in Europe. With time, Refinement has evolved from its original consideration during animal use to an all-encompassing concept to be taken into account before, during, in between and after experiments; a continuous Refinement is a legal obligation during use as well as in all breeding and care practices.

The talk will discuss the key elements and infrastructures that provide the right setting to implement a continued Refinement in all interaction with animals. The developed Severity Assessment Framework Guidance provides tools on how to consider Refinement from the project design to day-to-day application and follow-up. The guidance for Animal Welfare Bodies and National Committees provide further ideas on how to build up the necessary support structures enabling animal welfare to remain a central focus in all care and use of animals.
Focussing on severe suffering
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In 2010, the RSPCA began working with the scientific community to develop and promote new approaches to help reduce the number of animals experiencing the highest level of suffering in research and testing (e.g. USDA category E).

As a scientific animal welfare organisation with a high level of liaison with scientific and regulatory communities, we have established a well-supported programme of work that has to date included:
- A comprehensive web resource to help the research community address severe suffering: www.rspca.org.uk/severesuffering.
- Downloadable guidance to help establishments through the process of reducing severe suffering.
- Five expert working group reports on reducing suffering in specific procedures, e.g. sepsis and rheumatoid arthritis.
- An International “Focus on severe suffering” meeting in Brussels in 2016.

This talk will provide more information on our severe suffering resources and explain how we work with the scientific community.

References
Hawkins et al. (submitted). Applying refinement to the use of mice and rats in rheumatoid arthritis research.


Establishment of a Global Pharmacology Council to optimize and standardize in vivo models and secure full integration of the 3Rs in decision making processes in a global research unit
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At Novo Nordisk we have 3 global sites that work with in vivo pharmacology.

In order to ensure cross site quality and reproducibility, we have established a Global Pharmacology Council (GPC) which reports to the Global Research management team.

The GPC has governance of all in vivo models and technologies (model catalogue) used to create data for milestone passages of new drug candidates. The council is responsible for ensuring global quality and reproducibility and drive that learnings are shared globally. Furthermore we strive to have all models assigned to a centre of excellence to ensure state of the art animal research.

All new models have to be approved by the GPC which evaluates the unmet need, scientific rationale, translational value and the 3Rs. The GPC guides in the establishment and characterization of a model including definition of success criteria. With a positive conclusion on a new model, the model enters the model catalogue.

Learnings from the past 2 years, especially from a 3R perspective, will be presented and discussed.

Rehoming of laboratory animals: Local policy and dilemmas in an academic setting
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Directive 2010/63/EU contains provisions for the rehoming of former laboratory animals and the conditions under which this can occur. These terms and conditions are incorporated in the Dutch Experiments on Animals Act (Wod). For animals that have been used or were intended for use in an animal procedure rehoming can be an option provided the condition the animal allows it, there is no danger to public health, animal health or the environment and when appropriate measures have been taken to ensure the welfare of the animal. In 2016, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) has issued an Opinion on the rehoming of laboratory animals as well as specific Codes of Practice on the rehoming of cats, dogs and non-human primates.

Although Utrecht University and the University Medical Centre Utrecht (UMC Utrecht) had a rehoming policy already for years, this momentum was used to critically review the existing local policy. UU and UMC Utrecht not only house many different animal species, but also animals with a different status: in addition to laboratory animals also animal patients and animals used for farming are housed, often in the same facilities. Many of these are taken care of by the same professionals. Different legal regimes apply to these categories of animals. This can result in different conditions for housing, care and monitoring.

Especially in cases when a choice has to be made between re-use, euthanasia, or rehoming of animals these differences often give rise to profound discussions between students, animal care takers, veterinarians and members of the AWB.

In this presentation, an outline is given of the UU/UMC Utrecht policy on rehoming of animals. The starting point for UU and UMC Utrecht is that for surplus laboratory animals rehoming should be considered as a serious option. In project proposals and work protocols, applicants should indicate whether rehoming is possible and provide a reason when stating that it is not. The policy of UU/UMC Utrecht aims at creating conditions that foster a positive attitude and make rehoming easier and more successful. At the same time, it should provide for a structured and transparent framework, including a set of pre-conditions, which have to be clear and discussed before an experiment starts that includes animals for which rehoming may be an option. For instance, it must be clear already before an experiment starts how rehoming is operated and rehoming of cats, dogs and non-human primates.

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**Session VIII-2: Advances in Technology to Enhance Animal Welfare**

**Co-Chairs**

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Paul Schroeder, Animal & Plant Health Agency, Addlestone, United Kingdom

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**VIII-2-731**

**Bringing behavioral management together with instrumentation, a transformative technology for animal-centric care**

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The combination of instrumentation and behavioral management provides a transformative technology which presents a broadly accessible tool for caregivers to positively impact the animal experience. Behavioral management programs for animals are well-established and essential in providing opportunities to express species typical behaviors as well as circumstances to make choices or exercise control over their environment. NHPs can be trained using positive reinforcement to voluntarily cooperate with necessary clinical care. Likewise, invasive and or stressful handling can be limited by expanding training to incorporate instrumentation. Medical devices designed to reduce burden to patients have demonstrated meaningful improvements in health-related quality of life and are often adaptable for animal-centric application. The deliberate merging of technologies improves animal well-being and maximizes the likelihood of accurate translation of experimental data to the clinical condition.

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**VIII-2-116**

**Refining dog care: Evidence-based refinements to improve dog welfare and data output**

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The dog is the most commonly used non-rodent species in the safety assessment of new chemical entities (> 100,000 pa) yet we know little about their welfare and impact of routine practices on welfare (Prescott et al., 2004). While there is broad desire to implement effective refinements to many aspects of dog use there are barriers to uptake, including lack of evidence and resources specific to the research environment, and concerns about interference with data quality and study outputs. From our collaborative project across UK industry (http://www.refiningdogcare.com), we present evidence-based resources for good practice and a number of protocols will be shared. Techniques to improve welfare and data output, and prepare dogs for study life will be presented. These include facility and home pen design (Scullion Hall et al., 2017), enrichment (Hall, 2014), training (Scullion Hall and Robinson, 2016), predictability (Scullion Hall et al., submitted), handling and dosing techniques (Hall et al., 2015). We describe empirical evidence demonstrating both welfare benefits and ease of implementation of an effective training protocol for laboratory-housed dogs are described. Our welfare assessment framework (Hall et al., 2015) is employed to monitor the impact of planned Refinements on welfare, and to evaluate preparation for procedures.

**References**


Dosage matters – Tramadol applied via the drinking water for pain management in bone-linked mice models

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An alternative to repeated injections is the application of analgesics via the drinking water. However, studies that address the efficiency of Tramadol in the drinking water are scarce. Different recommendations exist regarding the dosage, and they widely differ from potentially under- to overdosing. We performed a refinement study embedded in a basic research study in the mouse osteotomy model evaluating two commonly used pain management protocols, Tramadol (two concentrations) and Buprenorphin in the drinking water, for their efficiency and side effects on experimental readout in a mouse osteotomy model. We monitored (i) general parameters of wellbeing e.g. MGS, clinical scoring, weight, water, food uptake and (ii) model specific pain parameters. Our results show that high dosage of Tramadol can lead to sedation and reduced wellbeing compared to an effective but lower dose or buprenorphine treatment. No side effects on bone healing read-outs (µCT, histology) occurred.

Refinement of repeated arterial blood sampling in pigs

Vladimir Bubalo, Iris Wiederstein-Grasser and Birgit Reininger-Gutmann

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Preclinical in vivo studies continuously testing glucose sensors over a longer period of time require frequent blood sampling to verify the sensor readings. Repeated arterial blood sampling in pigs is challenging due to their predisposition to vasospasm and vessel rupture (Smith and Swindle, 2008; Wolfensohn and Loyd, 2006). The cannulation of the saphenous artery, which is routinely used during anaesthesia, does not provide safe access for frequent collection of bigger blood amounts, because of the smaller diameter of the catheter and risk of collapse of the vessel. In order to improve the ease and quality of frequent blood sampling and to further increase the number of sensors simultaneously tested per pig, a four-sided central venous catheter was placed into the A. carotis communis and Arteria femoralis sinistra et dextra. This method allowed simultaneous testing of 8 glucose sensors and successful blood sampling over 14 hours without any complications. Thus a 75% reduction of animals needed can be expected in this kind of studies.

References


Development of non-lethal sampling methodology to investigate salmonid host immune responses to ectoparasites

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Recently fish immunologists developed approaches for individual fish monitoring to reduce fish use, especially in disease studies. To date the focus has been viral and bacterial pathogens but many health issues are due to ectoparasites. Hence, we have established an individual monitoring methodology for amoebic gill disease, caused by the parasite Neoparamoeba perurans. To ensure data from individually monitored fish are representative of natural disease progression, the effect of repeated anaesthesia on both parasite and host was assessed. After selecting AQUI-S® as appropriate anaesthetic, post-smolt salmon were PIT-tagged and challenged (2500 amoeba/L). Comparative analysis of gill scores confirms that repeated gill swabs do not alter disease progression. Gene expression of non-lethal samples mirror upregulation in lethally obtained tissues. In summary these samples convey immunologically valid data, highlighting the potential of improving on 3R’s in aquaculture disease research.
Ultrasound and infrared thermography as a non-invasive technique to investigate mastitis in sows

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Udder diseases in sows are of large economic importance characterized by reduced milk and high mortality of the piglets. The purpose of this study was to compare the clinical inspection and palpation of the udder with results of the examination by ultrasound and thermography.

Examination was performed in 107 sows. Thermal images were taken from both sides of the sow picturing all mammary glands. Ultrasound scans were done cranial, caudal and at both sides of the teats of each mammary complex.

Results show that chronic mastitis, which was not detected by clinical diagnostics, could be detected by ultrasound control. Using thermography, these alterations could be illustrated by measuring a decrease in the average surface temperature of the affected area in comparison to the whole complex. The results contribute to improve the diagnostic of udder alterations of sows by using non-invasive imaging techniques as an additional diagnostic tool to classical examination (Refinement).
**Session VIII-3: Establishing a Culture of Care Through Assessment, Transparency, and Communication**

**Co-Chairs**

**Gregory Reinhard**, University of Pennsylvania, Philadelphia, PA, United States  
**Yumiko Kirihara**, Shimane University, Matsue, Japan

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**VIII-3-725**  
**Culture of care – Thoughts and processes to enhance it**  
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An institution with a positive culture of care for laboratory animals is common goal. This presentation will examine some philosophies, methodologies, and practical approaches strengthen scientists’ and animal care staff’s caring culture for laboratory animals.

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**VIII-3-19**  
**A culture of care: Animals, people and communication**  
**Bella Williams**  
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Public acceptability of animal research, reflected in current legislation, is conditional on both the scientific justification, and the care that is taken of the animals. While facilities place much-warranted emphasis on providing animal care and welfare through the 3Rs, the recent idea of a “culture of care” implies integrated practices that ensure both care of the animals and care of facility staff.

Good science depends on good animal welfare practices, but if staff are to care for their animals effectively then a working environment that values and fosters caring practices is also vital. To help develop more formalized practices around care, UAR has worked with technicians, researchers and stakeholders, mapping their experiences map to existing social and ethical frameworks of caring and care-work. A clearer understanding of care as an organizational value and working practice, will allow institutions and individuals to take steps that improve both staff and animal welfare. The framework presented here supports strategic steps towards recognizing and building caring practices within laboratory animal facilities.

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**VIII-3-252**  
**A practical example of measuring culture of care**  
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Novo Nordisk has developed a tool to measure the company’s culture of care using surrogate markers. Surrogate markers are components that relate to culture of care – the way we behave and the way we think in relation to our work with laboratory animals. The surrogate markers are: three main top-level themes that each has a value-based characteristic – collaboration, trust and integrity, and six operational topics – influence on job situation, meaning of the job, predictability in particular situations, social support, rewards or recognition related to the job and resources to do the job. The survey looks at four different levels: the individual employee, the single groups working with the animals, the management’s role and also organisational structures.

The measuring tool is a quantitative survey and it assesses the state of the company’s culture of care and it identifies potential gaps. The gap analysis is followed up by qualitative interviews which are essential to initiate action plans in order to assure optimal animal welfare in terms of Reduction and Refinement.

The preliminary pilot study is presented and an outline of potential actions is described.

**Reference**  
Directive 2010/63/EU

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**VIII-3-685**  
**Delivering a good culture of care**  
**David B. Anderson and Susanna Louhimies**  
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A multitude of factors require careful consideration if an effective culture of care is to be delivered. The challenges differ dependent on the nature of the establishment. In all circumstances, there needs to be commitment and support from senior management to ensure appropriate resource and personnel are available.

The structure and processes in place also need to be kept under continuous review to ensure the establishments remain abreast of new innovations in animal welfare, care and use and to ensure effectiveness.

The presentation will explore the various challenges in delivering an effective culture of care, consider how these may be overcome and the opportunities for the different roles to actively contribute to it. Finally, a whole institute-embracing culture of care provides the right environment for a constructive culture of challenge to be established, benefitting both the science and the animals.
**Session VIII-4: Ensuring Good Welfare for Genetically Engineered Animals**

**Co-Chairs**
- Boris Jerchow, Working Group of Berlin Animal Welfare Officers and Medical Center Hamburg-Eppendorf (UKE), Germany
- David Anderson, DG Environment, European Commission, Brussels, Belgium

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**VIII-4-546**

**Application of the three Rs in creation and breeding of GA animals**

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The use of Genetically Altered (GA) animals in scientific procedures continues to increase year on year, indeed in some countries the use of GA mice exceeds the use of conventional animals.

To ensure that the three Rs are effectively implemented, consideration has to be given at all stages of production and maintenance to ensure that the most effective processes and monitoring systems are in place to minimise numbers and degree of suffering.

The presentation will explore the frameworks necessary within establishments to ensure compliance with the Three Rs, how a consistency of approach can be encouraged and how common standards and practices can be encouraged nationally and internationally.

A consistent approach is also necessary to promote a common understanding of the impact of GA manipulation when reporting the severity of scientific procedures.

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**VIII-4-644**

**Respecting the 3Rs when using CRISPR technology to generate GE mice: Strategies and comments**

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New technologies for genetic engineering may have unintended effects upon the 3Rs requiring consideration. This is particularly relevant with CRISPR technology. For individual projects, CRISPR allows investigators to generate animals easily and cheaply. Thus, one can reduce animal numbers used for an individual project. However, because of the ease of the procedure, many more projects may be attempted, offsetting this reduction. As well, the components of the CRISPR system can be introduced into mice using methods other than standard pronuclear injection, thus providing reduction in animal numbers used and refinements in techniques that benefit the animal. Other reductions in animal use may be obtained by carefully planning genotyping strategies and choice of guide RNAs. Finally, assessment of CRISPR mutations in differentiated ES cells and the use of human cells may partially replace the use of whole animal models, or reduce the numbers of animals required to obtain the correct model.

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**VIII-4-418**

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

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Toe clipping and ear clipping are frequently used for the individual identification of laboratory rodents. These procedures potentially cause severe discomfort, which can reduce animal welfare and distort experimental results. Since no systematic summary of the evidence for discomfort due to toe or ear clipping in rodents currently exists, we performed a systematic review on this topic. We identified 7 studies on the effect of ear clipping on welfare-related outcomes, and 5 such studies on toe clipping. Study characteristics and outcome measures were highly heterogeneous, and there was an unclear or high risk of bias in all studies. Out of > 60 different outcomes, 3 indicated an effect of ear clipping and 4 an effect of toe clipping. In conclusion, the existing body of evidence is too small and of insufficient (reporting) quality to reliably assess the effects of toe or ear clipping. Adequately powered, high-quality studies reporting reliable, relevant outcomes are urgently needed.
Ultrasound confirmation of pregnancy in genetically modified mice reduces resource use while enhancing reliability

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We have implemented the use of ultrasound as a non-invasive, early and reliable means to confirm pregnancy in mice.

The mouse is widely used as a model to study embryonic development. The traditional way for assessment of pregnancy in mice is direct visual observation or abdominal palpation, though the reliability of these methods prior to E12.5 depends on the skill of the technician and is dependent on litter size. We have determined that only 60% of females that show evidence of mating are found to be pregnant at the time of ultrasound, allowing us to reuse the non-pregnant animals for other purposes. The ultrasound process involves anesthetizing animals with isoflurane, chemical removal of the abdominal fur, imaging the animals on a heated stage, and monitoring for recovery from anesthesia. The use of this method reduces the impact to our collaborators’ experimental timelines and conserves the complex mutant mice.

Implement of the 3R principles in genetic modified mice production

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Genetic modified mice (GM mice) are valuable models for biomedical research, especially in the field of investigating gene function, developmental biology, and diseases. The process of producing GM mice involved several main procedures, often includes superovulation, microinjection, embryo transfer and germline test. Both the production process and the induced mutation might have significant impact to the welfare of animals. In order to elevate the welfare status in GM mice production, we implement the 3R principles into each procedure.

In this report, we demonstrate after optimizing euthanasia methods, embryo transfer settings, animal identification and pain evaluation methods, we not only significantly decreased the number of animal used for GM mice production, and are able to recognize and minimize potential health problems due to gene modification.

Welfare assessment and severity classification of genetically altered rodents

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Genetically altered (GA) animals are frequently used research models with continuously increasing numbers. Apart from its scientific value, a genetic alteration can compromise an animal’s wellbeing. However, the large variety of phenotypes is challenging when it comes to welfare assessment and severity classification, which plays an essential role in pro- and retrospective severity assessment.

In Germany, national guidance has been developed on a basic welfare assessment and documentation of strain characteristics. Recently, the Working Group of Berlin Animal Welfare Officers devised an example driven guideline on how to classify different phenotypes into severity categories. The Guidelines on severity assessment and classification of genetically altered rodents contain examples of symptoms and syndromes caused by genetic alterations. Examples are assigned to a particular severity category (none, mild, moderate, severe) including recommendations for monitoring and refinement strategies. Beyond the borders of the European Union, this guideline will contribute to the harmonization of severity assessment of genetically altered mice and rat lines.

The presentation gives an overview about the idea of welfare assessment of GA rodents and demonstrates the approach of severity classification on the basis of examples.
Novel cage-side assessments of post-operative pain in mice

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The ability to rapidly and accurately identify pain in mice is critical for providing them optimal care and welfare. To meet this need, we developed and validated the Grooming Transfer Test (GTT) and Nest Consolidation Test (NCT). We assessed these novel tests along with electronic von Frey and ambulatory parameters at baseline, after iso-flurane anesthesia +/- analgesia, and after laparotomy in adult CD1 and C57BL/6 mice of both sexes, housed singly with or without an existing nest or in pairs. While ambulatory parameters had no and von Frey responses minimal significant changes after surgery, GTT and NCT were significantly altered for 48 hours after surgery in both sexes, strains, and across the various housing conditions. Buprenorphine and carprofen each reduced post-operative pain, however only the combination of the two completely prevented delays in nesting behavior. Therefore, these two novel cage-side methods can be used to quickly and objectively identify mice from a variety of signalments and housing conditions with alleviated and unalleviated postoperative pain.

Can we diagnose poor welfare at the cage-side? The need to validate welfare tests for diagnostic sensitivity and specificity

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Several techniques have been proposed as diagnostic tests of animal welfare (grimace scales, nesting and burrowing activity etc.), such tools should allow rapid, non-invasive and reliable identification of poor welfare so suffering animals can be treated (or humanely killed). Thus, these tests are analogous to clinical diagnostic tests and must be sensitive (reliably detect poor welfare) and specific (detect animals with good welfare). Some proposed tests do not fulfil these criteria. Tests unsuitable for cage-side use may have other uses (assessment of retrospective severity or the efficacy of interventions at group level etc.), but can pose risks if relied upon to diagnose poor welfare in individuals. If suffering animals are not detected due to low sensitivity (false negatives) they may go untreated, or be unnecessarily removed from studies and killed. False positives (low specificity) may lead to unnecessary treatment or euthanasia.

I argue there is a need for validation of potential cage-side welfare tests (including systematic review and meta-analysis) to establish their sensitivity, specificity and practicality before they are routinely used.
Preventing, recognizing and combating pain in laboratory animals

Marjolein Schilders-van Boxtel, Coenraad Hendriksen, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Wim de Leeuw, Pieter Roelfsema, Frauke Oli†, Frank Dales and Monique Paris

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In March 2015 the Minister for Agriculture (EZ) commissioned the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) to provide its opinion on the procedure and application of best practices to assist researchers and Animal Welfare Bodies (IvDs) in recognising and managing laboratory animal pain in the workplace, depending on animal species and nature of the animal procedures. At the heart of the advisory report from the NCad is the Code of Practice (CoP) “Prevention, recognition, and management of pain in laboratory animals”. This CoP was drawn up by a working group of experts and provides guidance to all parties involved in animal procedures in the prevention, recognition, and management of pain in laboratory animals.

Compelling arguments may be raised for not applying pain management. In response to these, NCad advises that the Central Authority for Scientific Procedures on Animals (CCD): 1) require researchers to provide properly supported arguments justifying the decision to ignore the issue of pain management; 2) focus attention on the non-pharmacological management of pain, for example by improving husbandry or the application of humane endpoints; 3) keep a register of arguments for not applying pain management, those arguments that are deemed valid, and the results of any additional studies commissioned by the CCD.

Furthermore, the advisory report includes the following recommendations to the Minister: 1) to commission a report into the objective assessment, standardisation, and validation of a pain-scoring system; 2) to make the relevant curriculum committees responsible for ensuring that existing training courses include sufficient focus on pain recognition and management, that the provision of continuous education with regard to pain recognition and pain management is where necessary updated in line with, and responds to, the identified need for visual learning material and e-learning modules; 3) to promote the creation of a network of experts with a (inter)national centralised point of contact. In addition, NCad has taken the lead to bring about cooperation of a network of experts with a (inter)national centralised point of contact.

Defining lifetime use and cumulative endpoints for research animals

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Humane endpoints for animal studies are refinements and are considered to be the earliest time at which an experimental animal’s pain or distress can be avoided or ended by taking actions such as providing euthanasia, relieving pain or terminating the study. Along similar lines, cumulative endpoints may be considered for animals used in more than one protocol for an extended period of time (i.e., lifetime use) or in individual protocols that involve multiple procedures conducted over an extended period of time. We surveyed individuals working in research settings around the world regarding whether and how lifetime use and cumulative endpoints are being tracked and evaluated by Animal Ethics Committees at different institutions, and for which species. Over 150 responses were received, the majority coming from academia, industry, and government. While most Animal Ethics Committees have established formal endpoint policies for experimental use that cover many (although not necessarily all) research species, almost no facility has developed endpoint policies related to the issue of pain.

Compelling arguments may be raised for not applying pain management. In conclusion, the severity of both single and repeated isoflurane anesthesia in C57BL/6J mice can be classified as mild when adhering to our anesthesia protocol. However, within the mild severity category, repeated anesthesia ranks higher than single anesthesia, with female mice being more vulnerable than male mice.
VIII-5-411

Studies on the anesthetic effects of a mixture of medetomidine, midazolam and butorphanol, and antagonism by atipamezole in small rodents

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A mixture of medetomidine (MED), midazolam, and butorphanol has been recently used for as an injectable anesthetic in mice and rats instead of the more common ketamine using in Japan. This mixture produced a sufficient anesthetic duration of about 40 minutes in ICR, BALB/c, and C57BL/6 J mice strains by intraperitoneal (IP) injection (Kawai et al., 2011; Kirihara et al., 2013). We also assessed the anesthetic effects of the mixture administered by subcutaneous (SC) and intravenous (IV) injection compared to IP administration. We found that SC injection of the mixture worked equally as well as the IP injection (Kirihara et al., 2015). We compared the effects of the mixture using three different rat strains. We then found that the mixture produced almost the same effects in the rat strains (Kirihara et al., 2016). Atipamezole (ATI) can antagonize an effect of MED. After administration of the mixture, an injection of ATI made the mice and rats rapidly recover from anesthesia (Kirihara et al., 2015, 2016). During the experiment, we measured vital signs using a pulse oximeter. The results may indicate that the anesthetic mixture is an effective anesthesia for laboratory mice and rats. Also, this study may contribute to the welfare of laboratory animals.

References

Session VIII-6: Training Animals for Better Science and as Partners in the Scientific Process

Co-Chairs
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VIII-6-577
Positive reinforcement training for research primates to improve animal welfare and research quality

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There has been a revolution in care for nonhuman primates in research and testing facilities, and the widespread application of positive reinforcement training methods has been part of this change. Positive reinforcement training is a refinement in animal handling methods that can improve animal welfare, animal husbandry, veterinary care, and research quality. Using positive reinforcement training methods primates are taught to voluntarily cooperate with procedures rather than relying on coercion. They can be taught to cooperate with a variety of procedures that are a routine part of life for research primates including moving between enclosures, allowing examination of parts of their bodies, cooperating with the collection of biological samples (e.g., urine, vaginal fluid, blood) or with receiving injections, and calmly tolerating restraint. Positive reinforcement training can also be used to reduce aggression, fear and abnormal behavior in some cases.

VIII-6-653
Improving cat welfare through better handling

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Many cats display fear and aggression during handling, which can negatively affect welfare, and lead to inadequate physical exams and test results. In response to these concerns, various veterinary organizations are recommending changes to improve cat handling, including managing the environment to minimize potential stressors, and adapting handling by minimizing restraint through alternatives. However, the majority of these recommendations have yet to be assessed scientifically. Our current research is aimed at objectively assessing and improving handling methods for cats to reduce stress during exams and procedures. We will present recent results from a series of studies validating indicators of handling-related stress in cats, and assessing the effects of different environmental changes and handling techniques on cat responses. While the main aim is to improve cat welfare, these results also have the potential to improve research results by reducing stress-related variability.
**VIII-6-724**

**A lifetime of training for dogs in a research environment**

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A quality program for acclimation and socialization of dogs used for research, enhances the welfare of the dogs and staff, facilitates the work and improves scientific outcomes. This is accomplished through effective training programs and acclimation to the study, staff and facility. Optimization of restraint and the study environment improves the animal experience and well-being. Provision of appropriately configured caging and exercise spaces will allow for normal dog behaviors and social interaction. This will allow a smooth transition in the life span of the dog from puppy to the potential opportunity for adoption to a new home (rehoming).

**VIII-6-395**

Rehoming of former laboratory animals

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The aim of the advisory report “Rehoming of former laboratory animals” by the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) is to guarantee the quality of life of non-human primates (NHPs), dogs and cats that remain alive at the end of an animal procedure. The terms “putting up for adoption” and “retirement” are often used for such situations. In its advisory report, the NCad uses the term “rehoming”, by which is meant that, remaining alive at the end of an animal procedure, an animal is able to spend the rest of its life at a location suitable for its needs without being subjected to any further animal procedure. Based on the viewpoint that these animals have intrinsic value and should therefore always be treated as sentient beings, one should assume that all dogs, cats and NHPs kept alive (the “yes, unless” principle) are being rehomed.

Various options exist in the Netherlands for rehoming a former laboratory animal. Establishments offering these opportunities develop a lifetime of training for dogs in a research environment a framework that their own guidelines and procedures. To establish a coordinated and transparent rehoming process, NCad has drawn up a uniform standard to as large a population as possible. Applying a uniform standard to as large a population as possible has largely focused on identifying best practices to be applied at a species- or strain-level. E.g. mice should be provided nesting material and macaques should have fully access to appropriate social partners.

**VIII-6-737**

A personalized medicine approach to behavioral management

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As environmental enrichment programs have grown in both complexity and ubiquity, the research supporting enrichment decisions has largely focused on identifying best practices to be applied at a species- or strain-level. E.g. mice should be provided nesting material and macaques should have fully access to appropriate social partners. Applying a uniform standard to as large a population as possible has been mostly successful, and indeed makes enrichment in large institutions possible. However, less attention has been paid to determining which subgroups or individual animals may benefit from behavioral management strategies other than the “standard.” This presentation will raise examples from the literature of populations that appear not to benefit from the “one size fits all” approach, and how approaching behavioral management from a personalized medicine perspective can help improve the welfare or these animals.

**References**


More than 3Rs – The 3Vs to improve the validity and reproducibility of animal research

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The 3Rs concept serves to minimize harm in research animals. Whether the use of animals is justifiable, however, depends also on the expected benefit of the research. Unless study outcomes are valid and reproducible, animals may be wasted for inconclusive research. Accumulating evidence indicates risks of bias caused by flaws in the design and conduct of animal research. I therefore propose a more systematic assessment of scientific validity when reviewing grant proposals, study protocols, and publication manuscripts, including evidence of construct validity, internal validity, and external validity of the expected outcomes. As with the 3Rs, there is no need for a fixed checklist approach. Instead, criteria for assessing the 3Vs could be defined according to the decisions to be taken (e.g. project funding, protocol approval, publication). Together with the 3Rs, the 3Vs would thus help to avoid wasting animals for inconclusive research and imposing unnecessary harm on research animals.

PREPARE guidelines for planning animal research and testing

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In attempts to improve the validity, reproducibility and translatability of animal experiments, a number of reporting guidelines have been written. There are, however, many other factors, seldom reported in the scientific literature, which can influence the outcome of experiments, animal welfare, and the health and safety of all concerned. We have produced guidelines for planning animal experiments, called PREPARE (Planning Research involving Experimental Procedures on Animals: Recommendations for Excellence). PREPARE covers all stages of quality assurance, from the management of an animal facility to individual procedures. They are also relevant to field experiments. More information is available on the PREPARE website: https://norecopa.no/PREPARE.

An omics based approach to improving reproducibility in animal-based studies

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The reproducibility of in vivo research has been attributed to a lack of multiple factors including: environment, genetics, microbiome, technique, bias, statistics, and adequate reporting. Controlling all of these variables is not feasible or practical. By adapting an omics-based approach, reproducibility in animal studies could be greatly improved. In these omics studies, standard housekeeping biomarkers are measured for quality assurance and as comparators with each data set. In adapting this strategy to in vivo studies, these biomarkers could be physiologic, metabolic, cellular, or molecular in nature (or any combination thereof), and ideally sampled prior to initiation (baseline data) and longitudinally. Examples will be provided. By adapting this strategy to in vivo studies, greater context of the system’s microenvironment would be provided, interpretation of results would be facilitated, and outcomes could be more readily standardized across different laboratories.

Global enrichment challenges for non-human primates: From the lowest to the highest hanging fruit

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All primate facilities face challenges in their enrichment strategies. Where there is little historical experience of fine-tuned enrichment plans, animal housing can tend towards the basic. This leaves small, typically inexpensive, enrichments (often widespread in Europe and N America) available to improve welfare substantially. In more mature contexts, the lower hanging fruit have often already been picked. Here challenges persist that can be hard to meet, including keeping enrichment strategies appropriate and novel in the face of evolving regulations and best practice recommendations. To improve global standards we must encourage those yet to pick the low-hanging fruit to do so successfully and safely, rather than requiring all fruit to be picked at once. This pragmatic approach builds a sustainable enrichment culture that recognises the positive impact of effective enrichment, while addressing the reproducibility challenges resulting from globally varying enrichment standards.
Gut microbiota and animal model reproducibility

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The gut microbiota is composed of up to $10^{14}$ cells and their various metabolites living, dying, and reproducing throughout the gastrointestinal tract of mammals. It co-evolved with each host species to assist with day to day functions, contributing to the overall health of animals in remarkable ways. Because of the significant beneficial impact that gut microbiota may have on other organ systems there is interest in learning more about the gut microbiota and translating these findings into clinical therapies. Results from recent studies characterizing the gut microbiota have demonstrated that many factors may affect gut microbiota diversity. Relatively little is known about the functional consequences of alterations of the gut microbiota and exactly how changes in richness and diversity of the microbiota result in changes in health and susceptibility to disease. Questions have also been raised as to whether ultraclean, barrier-raised mice are relevant models of human disease, given their reduced gut microbiota diversity and complexity. This talk will explore animal model reproducibility in light of new findings about the gut microbiota.

Characterizing the variability of LD$_{50}$ values in acute toxicity methods: Implications for alternative methods development

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In _in vivo_ LD$_{50}$ values are often used as reference data to evaluate alternative methods to estimate acute toxicity. However, to achieve a fair assessment of alternative methods, it is important to determine the extent to which _in vivo_ studies vary or predict themselves. We obtained LD$_{50}$ values from multiple databases, including the NLM’s Hazardous Substances Data Bank and ChemIDplus, the OECD’s eChemPortal, and the JRC’s AcutoxBase, yielding a total of 27,380 oral LD$_{50}$ values representing 11,276 unique chemicals and 13 species. All chemicals with ≥ 5 studies had variable LD$_{50}$s spanning at least one order of magnitude, with some ranging over four orders of magnitude, not only across rat studies but also across multiple species. These results underscore the importance of considering an appropriate margin of uncertainty when using _in vivo_ acute oral toxicity data for the assessment of alternative methods.

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A welfare assessment framework for understanding and improving welfare and data output in the dog

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Dogs are the most commonly used non-rodent species in safety assessment of new medicines with > 100,000 used per year. Previous research has identified a need to better measure welfare and the concomitant link with data quality in the laboratory-housed dog (Prescott et al., 2004). Crucial decisions relating to housing, husbandry and regulated procedures may be based on anecdotal rather than empirical evidence and effects of planned Refinements not measured to identify welfare benefits. We developed a Welfare Assessment Framework (Scullion Hall et al., in prep) that describes a system of measurement for welfare and data output including behaviour, affect, clinical pathology, mechanical threshold and cardiovascular output. Data will be presented from dogs (n = 200) housed in different units with contrasting histories of regulated procedures, housing, husbandry and training techniques. The Welfare Assessment Framework has been employed to examine areas in need of Refinement and devise Refinements to benefit welfare and data output, such as improved home pen and facility design (Scullion Hall et al., 2017), training (Scullion Hall and Robinson, 2016), dosing (Hall et al., 2015) and signalled predictability (Scullion Hall et al., submitted).

References


**Session VIII-8: Advances in Recognition and Treatment of Pain in Laboratory Rodents**

**Co-Chairs**
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**VIII-8-747**

**Pain assessment and new innovations**

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For many years animal care workers have been striving to refine the assessment of pain in rodents by scoring behavioural and visible characteristics indicative of discomfort. Being able to focus on particularly relevant times such as post-surgery or after treatment facilitates early intervention and amelioration. However, where the source of the pain is present throughout the lifespan of the animal, but may manifest itself sporadically and unpredictably, it is still very difficult to provide effective management. This is particularly the case with genetically altered (GA) animals carrying debilitating mutations or of newly generated GA strains where the phenotype has not yet been characterised.

To overcome the constraints of manual observations including long periods of assessment time, disturbing animals from their usual environment and lighting conditions, it is necessary to develop new ways of assessing pain.

Home cage monitoring equipment is being developed to record laboratory rodents 24 hours a day, in their home cages and throughout dark periods. This will greatly facilitate the detection of different patterns of activity and social behaviours that will serve as early indicators of pain and discomfort, thereby facilitating early intervention and care.

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**VIII-8-716**

**Preventing and alleviating pain associated with experimental procedures in laboratory animals**

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The effective alleviation of procedure-related pain in laboratory animals is an important goal. Despite the emphasis given to humane treatment of laboratory animals in the national legislation of many countries, analgesics may still not be administered routinely in the post-operative period. This omission is particularly common in studies using small rodents.

Early misconceptions about the nature of animal pain limited effective pain management in all species, but this problem has now largely been overcome. The risk of clinically significant side-effects of analgesics has also limited their use, but this concern has reduced as our understanding of species differences in the activity of analgesics has increased. A wide range of different analgesic agents are available, and many of these underwent preclinical assessment of efficacy and safety in small rodents. Potentially safe and effective analgesics are therefore available. However, we still lack data on the clinical efficacy and duration of action of many of these agents. This is reflected in the wide range of doses reported in the literature, and the widely varying duration of treatments. The potential role of novel products, such as slow-release formulations and techniques such as epidural and intrathecal routes of administration have also not been properly evaluated. Finally, analgesic use rarely involves multimodal therapy, and structured therapeutic approaches analogous to the WHO Pain Ladder are not used.

It is also necessary to incorporate pain assessment and management into an overall scheme of perioperative care. We need to be concerned with distress, as well as pain, and should be aware that practices such as handling methods can significantly influence animal stress, as can anaesthesia, intraoperative care, and postoperative management. Increased stress can increase pain and reduce the efficacy of analgesics. Attention to all of these factors is necessary if we are to refine research procedures effectively.

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**VIII-8-586**

**Effects of analgesics and pain on research outcomes**

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A major challenge faced by scientists and bioethics review committees in evaluating several research proposals is in predetermining how unrelied pain or the choice of analgesics can influence outcomes. There tends to be a bias toward the negative impact of the latter, and this is likely the result of the perception that the addition of pharmaceuticals adds unknown variables and that efforts needed to treat and monitor pain have little scientific value. Consideration in the assessment of requests for exemption to the use of analgesics will be discussed. Several studies demonstrate that the molecular and physiologic effects of unalleviated pain upon the model need to be considered and that lack of intervention may adversely affect outcomes. Additionally, the translational relevance of the models must be considered when the use of analgesics is questioned. The information provided here will help scientists and reviewers make sound decisions on the use analgesics in *in vivo* studies.