

**LASA 3Rs Section / UFAW Meeting**  
27<sup>th</sup> September 2022, GSK Stevenage

**Stitching the 3Rs into surgical procedures**

**Programme**

- 09:20-10:20 Registration and coffee
- 10:20-10:30 **Welcome and introduction**  
*Session one chair - Joanna Moore, GSK*
- 10:30-11:00 **Cost effective and safe industrial scale extra warming system for IVC-cage maintenance of health compromised mice**  
*Jukka Puoliväli, CRL*
- 11:00-11:30 **Refinements to the use of analgesia/anaesthesia and routine surgical approaches**  
*Sarah Chadwick, SLC Veterinary Services*
- 11:30-12:00 **Applying the principles of good experimental design**  
*Esther Pearl, NC3Rs*
- 12:00-12:30 **Analysis of refinements in surgical procedures: status quo and potential for improvement**  
*Paulin Jirkof, University of Zurich*
- 12:30-13:30 ~ Lunch ~  
  
*Session two chair - Emma Stringer, University of Birmingham*
- 13:30-13:55 **Application of the 3Rs for minipig safety pharmacology: anaesthesia, surgery and colony management**  
*Kate Read, Labcorp*
- 13:55-14:20 **Identify surgical recovery via locomotion: insights from experimental data via digital ventilated cages**  
*Stefano Gaburro, Tecniplast S.p.A*
- 14:20-14:45 **Refining the unilateral ureteral obstruction mouse model: no sham, no shame**  
*John Atkinson, UCB BioPharma*
- 14:45-15:00 Final comments and close of meeting

*LASA has awarded this meeting 5 CPD points*



*This meeting is supported by  
Laboratory Animal Veterinary Association*

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**Stitching the 3Rs into surgical procedures**

## Abstracts

### ***COST EFFECTIVE AND SAFE INDUSTRIAL SCALE EXTRA WARMING SYSTEM FOR IVC-CAGE MAINTENANCE OF HEALTH COMPROMISED MICE***

*Jukka Puoliväli<sup>1</sup>, J Semi<sup>1</sup> and T Heikkinen<sup>1</sup>*

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Charles River Discovery Research Services, Kuopio, Finland site performs preclinical studies with rodent models of neurological disorders. In many of these models, and mouse models in particular, animals might suffer from hypothermia that can be fatal. Extra warming has been shown to mitigate the loss of core body temperature for example in an MPTP mouse model of Parkinson's disease and improve the wellbeing of animals. However, it is difficult to provide additional warming in research settings for mice housed in IVC cages, safely and reliably overnight without monitoring.

A warming system using reptile heat cables was developed and manufactured on site and installed underneath the IVC cages. Prior to use, pilot studies were conducted to ensure that the system worked safely, and that overheating was not possible. Temperatures were recorded for 24h via a Vaisala recording device placed in cage on the bedding with no heat cables, and with 50W and 70W heat cables. Temperature recordings were performed with an empty cage and with a cage including 4 mice.

In an empty cage without heat cables the inside temperature was 23.3 °C. When using 50W and 70W heat cables in an empty cage, the internal cage temperature was 25.5 °C and 27.2 °C, respectively. When the similar temperature recordings were conducted with 4 mice, the internal temperature was 23-24.5 °C when no heat cables were used, and 26-28.5 °C with 50W and 27-29 °C with 70W heat cables, respectively. In summary, the use of 50W heat cables increased the internal cage temperature by 2-4 °C, whereas 70W heat cables resulted in an increase of 3-6 °C in a cage with 4 mice.

Reptile heat cables placed underneath IVC cages proved to be an easy, safe and cost-effective way to provide extra warming for mice. Heat cables can be left overnight without danger of overheating or fire hazard. Use of extra warming improves the well-being of hundreds of mice used at the site yearly. The retrospective review of historical data from completed studies in an MPTP model has shown that model-related mortality has decreased from 5.9% to 1.9% since implementation of extra warming. There is also evidence suggesting that extra warming could decrease mortality in other models as well. This project has received a Charles River 3Rs award.

### ***REFINEMENTS TO THE USE OF ANALGESIA/ANAESTHESIA AND ROUTINE SURGICAL APPROACHES***

*Sarah Chadwick, SLC Veterinary Services*

Admiral Grace Hopper famously said "The most dangerous phrase in the language is, 'We've always done it this way'".

For those of us performing surgery, there is a risk of us becoming accustomed to certain techniques and approaches, that whilst effective, no longer represent the most appropriate or refined approach. Whilst we all want the best for the animal and the scientific outcome, when it comes to changing surgical approaches, implementing change can feel daunting and high-risk in case something goes wrong.

This talk will present widely applicable and relatively simple approaches that are either new or under-utilised as surgical refinements, with the aim of promoting subsequent local discussion.

Topics will include the use of intradermal sutures as a more refined alternative to wound clips or traditional sutures (both of which are associated with different welfare concerns), and the latest choices of anaesthesia available in the pre-clinical setting. This includes examples where systemic anaesthesia is used routinely, but a local anaesthesia would offer a better alternative as it reduces the depth of anaesthesia making it safer, and provides better analgesia in recovery. The talk will also introduce a selection of anaesthetic drugs available and demonstrate a range of methods by which they can be used based on experience in both an experimental and clinical setting

### **APPLYING THE PRINCIPLES OF GOOD EXPERIMENTAL DESIGN**

**Esther J Pearl**

**National Centre for the Replacement, Refinement and Reduction of animals in research, UK**  
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Many scientists and organisations are concerned that biomedical animal studies are not always reliable or predictive. Poor experimental design, inappropriate analysis methods and incomplete reporting contribute to problems with quality and reproducibility of animal research. In my presentation I will focus on key principles of good experimental design and provide tips to implement these in experiments, with particular emphasis on blinding/masking, which is used to prevent our expectations from unintentionally influencing experiments. I will cover the different stages of an experiment that can be blinded/masked, common barriers preventing researchers from using blinding/masking in their experiments and strategies to overcome these.

I will also highlight resources to help researchers improve experimental design and reporting. These include the NC3Rs Experimental Design Assistant (EDA, <https://eda.nc3rs.org.uk>), and the ARRIVE guidelines ([www.ARRIVEguidelines.org](http://www.ARRIVEguidelines.org)).

### **ANALYSIS OF REFINEMENTS IN SURGICAL PROCEDURES: STATUS QUO AND POTENTIAL FOR IMPROVEMENT**

**Paulin Jirkof**

**Office for Animal Welfare and 3Rs, University of Zurich, Switzerland**  
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Surgical interventions are ubiquitously used in biomedical research using mice for generating disease states, sampling tissue, implanting, or testing medical devices. Surgeries always may carry the risk of inducing more than short term post-surgical pain and suffering in the animals involved. The use of best-practice surgical techniques, peri-surgical pain management as well as the implementation of other pharmacological and non-pharmacological mitigation measures is therefore essential.

In a series of publications the status quo of refinement use (or better its reporting) in typical surgical interventions like embryo transfer, osteotomy and telemetry implantation surgery was analysed. Depending on the field reporting of surgery details and refinement measures was low to moderate. Almost no details for embryo transfer surgery are reported in an overwhelming number of publications describing models including this procedure. Publications describing osteotomy models on the other hand show a high reporting accuracy for model-specific information on fixation methods and fracturing procedures, while reporting was more often insufficient in terms of mouse characteristics, hygiene monitoring/immune status of the animal, anaesthesia, and analgesia. Likewise, for telemetry surgery, while specific details on telemetric procedures are reported well, rarely any reporting of refinement of implantation surgery procedures prior (e.g., acclimatization), during (e.g., asepsis) or after (e.g., social housing) the surgery could be observed.

In this talk I will present our findings and will give recommendations for improvements of pro- and retrospective severity assessment, surgical techniques, perioperative pain treatment and good reporting. I will also discuss often neglected simple, non-pharmacological refinement measures.

**Application of the 3Rs for minipig safety pharmacology: anaesthesia, surgery and colony management**

**KJ Read, MRCVS MA VetMB CertAVP**  
Department of Animal Welfare and Veterinary Services, Labcorp, UK  
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Surgical implantation of indwelling devices to measure cardiovascular parameters are required in multiple species for the assessment of safety pharmacology. The minipig presents a number of unique challenges, both in terms of surgical implantation and colony management. Replacement, reduction and refinement are considered throughout the process, including:

- Device selection and surgical model development
- Anaesthesia and peri-operative care refinement
- Study planning and potential for re-use
- Device longevity, malfunctions, and possibilities for repair
- Long-term colony management

This presentation will form a basis for discussion of the application of the 3 Rs to a surgical model, along with the maintenance of a surgically implanted colony, with specific reference to the minipig. It will document the evolution of our telemetered minipig program and the decision-making process involved in implementing the refinements.

#### **IDENTIFY SURGICAL RECOVERY VIA LOCOMOTION: INSIGHTS FROM EXPERIMENTAL DATA VIA DIGITAL VENTILATED CAGES (DVC®)**

**Stefano Gaburro**  
**Tecniplast S.p.A., Buggiate, Italy**

Definition of surgical recovery is typically achieved via assessment of parameters (e.g. body weight, animal fur..) that are used to build a clinical score to establish the severity of a procedure. Depending on the severity level to which animals are exposed from a few days to a couple of weeks are needed for the animal to recover.

However, objective evaluation through automated systems could provide a better picture of the current animal welfare status.

Recent technological development in home-cage monitoring (HCM) has proven that such detailed and punctual evaluations are possible.

During this talk, we will present examples coming from experimental settings where the assessment of surgical recovery was possible using digital ventilated cages (DVC®) technologies.

#### **REFINING THE UNILATERAL URETERAL OBSTRUCTION MOUSE MODEL: NO SHAM, NO SHAME**

**John Atkinson<sup>1</sup>, Tania Boden<sup>1</sup>, Jean-Phillipe Mocho<sup>2</sup>, Tim Johnson<sup>1</sup>**  
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Fibrosis, as a common final pathway in many renal diseases, contributes significantly to the decline of organ function and to progression to end-stage renal disease. To establish therapeutic interventions that target fibrosis, animal models are essential. The most widely used model of renal fibrosis is the unilateral ureteral obstruction (UUO) model. Typically, the control for this model is a sham-operated animal. Sham surgery causes pain and distress to these control animals, and here we aim to show that there is no difference in the main read-outs of this model between sham-operated animals and non-operated C57BL/6J mice.

In five experiments, quantification of Picro Sirius Red stained collagen in the renal cortex did not show any difference between 15 sham and 25 non-operated individuals. A comparison of the regulation of genes involved with fibrosis did not show any difference between sham and non-operated groups at 21 days post surgery either.

We conclude that there are no significant differences between non-operated controls and sham animals with respect to collagen deposition and fibrosis pathways in the UUO mouse model.