



Meeting Report

3 Days for 3Rs 2023: Refinement, Reduction, Replacement

doi:10.14573/altex.2402121

The second edition of the Italian Platform on Alternative Methods (IPAM, www.ipamitalia.org) webinar series “3 Days for 3Rs: Refinement, Reduction, Replacement” was held in October 2023. As in the previous edition, three sessions were organized on three days, each focused on a single R.

The first day, chaired by **Cristina Maria Failla** (IDI-IRCCS) and **Augusto Vitale** (Istituto Superiore di Sanità), was focused on *Refinement*.

The session was opened with a presentation by **Urte Jaeh**, Charles River Laboratories, with the title “The (r)evolution in rodent health screening: Promoting sentinel-free programs.” As the use of laboratory rodents in biomedical research continues to be essential, ensuring the health status of these animals has become a key priority for facilities across the globe. Rodent health screening makes an essential contribution to research by monitoring the presence of infectious agents that can compromise the animal’s health and become a confounding factor. Urte Jaeh started with the most common methods of pathogen detection and proposed sentinel-free options that would save a great number of animals, the sentinels, while maintaining robust data and reliability.

A contribution on refinement in neuroscience was presented by **Orsola Rosa-Salva** from CIMEC with the title “Refinement in cognitive neuroscience studies with domestic chickens.” Domestic chickens are emerging as a central animal model in cognitive science and neuroscience, particularly for studies on the developmental origins of fundamental cognitive mechanisms. At the same time, knowledge of chicks’ social and cognitive development could also be useful for their treatment in the commercial sector. Some refinement procedures that can be employed for young domestic chicks in laboratory settings were described.

The final talk of the day was presented by **Zoe Windsor** from University College of London on “Practical refinements in cranial device husbandry for laboratory mice.” The last decade has seen a considerable increase in the number of *in vivo* experiments making use of rodent cranial devices. However, cranial devices, with their protruding metal, pose a risk to the rodent’s health as they can become entangled or caught on common cage items, which can lead to injury that may require euthanasia. Consequently, due to safety concerns, mice with these devices face considerable restrictions on environmental enrichment items. Nesting material is an easily applicable form of enrichment that is beneficial to welfare and safe for the mouse. Nesting materials assessed for safety at UCL were discussed, as well as the provision of other modified enrichment items to promote high standards of welfare for animals which often face challenges to their enrichment protocol.

The second day, again chaired by Cristina Maria Failla (IDI-IRCCS) and Augusto Vitale (Istituto Superiore di Sanità), was focused on *Reduction*.

The first talk was presented by **Julia Steitz**, Aachen University, on “Virtual reality and new media in laboratory animal science education.” At the RWTH Aachen, FELASA-accredited courses are provided at different levels of education. Here, a blended learning concept combining virtual reality (VR) components with classical learning contents, including e-learning modules, new media, and practical skills training, was implemented and evaluated. Procedures, such as anesthesia induction, test substance application, and blood sampling in rats, as well as the laboratory environment, were captured on 360° videos, and VR teaching/learning modules were used to better prepare participants for hands-on training (refinement) or as a complete replacement of a live demonstration (reduction). Throughout the courses, users’ experience of the VR modules was evaluated, and it was demonstrated that, despite little previous VR experience, participants highly appreciated the VR modules, showing the potential to enhance procedures and demonstrations. Interestingly, participants with previous experience in laboratory animal science agreed even more strongly with the VR potential to support the 3R principle than less experienced participants and endorsed its use for further educational purposes. The high acceptance of this innovative training format is encouraging the development of further alternative education media in the field of laboratory animal science.

The next presentation by **Irene Ruspantini**, Istituto Superiore di Sanità, was on “Optimal sample size to attain sound statistical results in animal studies.” The successful application of the 3Rs concept of reduction in animal experiments is the solution to a multifaceted problem. With a key focus on statistics, Irene Ruspantini discussed the main issues to be considered and possible strategies to define the optimal sample size to both minimize the number of animals and achieve robust results, ultimately ensuring high-level and trustworthy scientific research.

The last presentation of the day was held by **Luigia Trabace** from the Università di Foggia. Her presentation title was “The importance of sex in preclinical studies: Application of the 3Rs principle.” At present, single sex usage is most common in preclinical studies in biomedicine. Notwithstanding the bias in the use of animal subjects that do not reflect frequency differences in pathologies affecting both women and men, experimental procedures continue to be by default biased toward using male over female animals. However, and contrary to widespread preconceptions, it has been clearly demonstrated that females are not more variable



than male rats. The propensity to underuse females can be found in all types of studies, from research using cell lines to *in vivo* experiments. The major impediment to reversing sex bias in basic science is the consideration that performing experiments on both sexes is contrary to the 3Rs principle of reduction. Instead, this poses a serious risk of wasting animals, which certainly is contrary to the 3Rs principles. Preclinical researchers should strive toward a cultural and structural change in how experimental studies in animals are performed and should recommend that funding agencies, regulators, journals, and editors embrace this paradigm change to obtain a more appropriate, equitable, and translational body of knowledge.

The third day, chaired by **Francesca Caloni** (Università degli Studi di Milano) and **Francesco Nevelli** (Merck Group), was focused on *Replacement*.

Giulia Ranaldi, from CREA-AN, gave a presentation entitled “Confocal laser scanning microscope as a tool to investigate intestinal *in vitro* model.” The confocal laser scanning microscope (CLSM) represents an important tool to visualize living and fixed biological samples. CLSM is composed of an optical fluorescence microscope connected to a laser source and an integrated advanced software for image processing that creates highly defined images and 3D digital elaboration of the analyzed samples. Due to these characteristics, the CLSM has been widely employed, e.g., on intestinal *in vitro* models, such as the 2D human differentiated Caco-2 cell line or microfluidic chips, to study intestinal behavior following different stimuli or insults. Notably, confocal morphological analysis of intestinal microfluidic systems has shown their high degree of differentiation and functional development, demonstrating their suitability for *in vitro* research investigation.

Laura Gribaldo, active senior researcher at the JRC, during her presentation on “Teaching 3Rs: focus on replacement”, underlined that education and training are fundamental to driving progress in the development and uptake of the 3Rs and that it is necessary to find common European strategies to involve experts, university professors, and students sensitive to this topic. Giving high priority to curriculum change is the first step to creating an environment where effective change can take place. Developing and implementing a curriculum framework can be a complex process, requiring high-level support from the government, ministries of education, and education planners. Therefore, a bottom-up approach can be complementary, since it involves working with educators at universities and considering ways of incorporating the 3Rs into their teaching using different learning scenarios designed to suit a variety of different learning contexts. A group of key specialists in teaching the 3Rs, invited to the JRC to discuss future needs and ways to improve dissemination of the 3R principle, recommended investing in the continuous professional development of teachers and lecturers. To expand the number of teachers, “train-the-trainer” approaches and support by sharing content and materials for education has been considered most helpful. A network of educators, within each Member State, who can share ideas and resources on the 3Rs, would be beneficial. The proposed work will be instrumental to produce, deliver, and disseminate a set of tools for education harmonized across Europe.

The last speaker of the session was **Lena Smirnova**, Johns Hopkins University, with a presentation titled “Brain MPS: Can we replace animal models of learning and memory with NAMs?” Recent advances in brain organoids derived from human stem cells have the potential to model learning and memory *in vitro*, offering the possibility of introducing more complex endpoints into the battery of tests for developmental neurotoxicity or neurological disease. The term “organoid intelligence” (OI) defines this emerging multi-disciplinary field at the intersection of advanced microphysiological systems with multielectrode arrays as sensor systems and artificial intelligence (AI). OI aims at leveraging biological learning mechanisms in the *in vitro* brain-computer interface. The multidisciplinary R&D trajectory is focused on scaling up organoid production and integrating novel electrode arrays. Challenges include developing models through stimulus-response training and creating organoid-computer interfaces. OI involves a collaborative effort of multiple disciplines.

Approximately 80 people from research, industry, and animal welfare organizations actively participated in one or more of the seminars and gave positive feedback on the event.

*Augusto Vitale*¹, *Maurilio Calleri*², *Francesca Caloni*³, *Cristina Maria Failla*⁴, *Paola Granata*⁵, *Laura Gribaldo*⁶, *Urte Jaeh*⁷, *Michela Kuan*⁸, *Stefano Lorenzetti*¹, *Francesco Nevelli*⁹, *Giulia Ranaldi*¹⁰, *Irene Ruspantini*¹, *Orsola Rosa Salva*¹¹, *Lena Smirnova*¹², *Julia Steitz*¹³, *Luigia Trabace*¹⁴, *Zoe Windsor*¹⁵ and *Isabella De Angelis*¹⁶

¹Istituto Superiore di Sanità, Rome, Italy; ²LIMAV Italia OdV, Imperia, Italy; ³Università degli Studi di Milano, Department of Environmental Science and Policy (ESP), Milan, Italy; ⁴Istituto Dermatologico dell’Immacolata, IDI-IRCCS, Rome, Italy; ⁵Gruppo MAPIC – Aispec – Federchimica, Milan, Italy; ⁶Active senior, European Joint Research Center – JRC, Ispra, Italy; ⁷Charles River Laboratories, Sulzfeld/Grabfeld, Germany; ⁸Anti Vivisezione, LAV Italia, Rome, Italy; ⁹Merckgroup, Ivrea, Italy; ¹⁰Food and Nutrition Research Centre, Council for Agricultural Research and Economics, CREA-AN, Rome, Italy; ¹¹CIMEC Centre for Mind/Brain Sciences, Università degli Studi di Trento, Rovereto, Italy; ¹²Center for Alternatives to Animal Testing, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; ¹³Aachen University, Aachen, Germany; ¹⁴Department of Clinical and Experimental Medicine, Università di Foggia, Foggia, Italy; ¹⁵University College of London, London, United Kingdom; ¹⁶formerly Istituto Superiore di Sanità, Rome, Italy