Meeting Report

Recent Advances in 3Rs and Laboratory Animal Science: Report on the International Conference of LASA (India)

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Introduction

This article summarizes the outcome of an international conference of Laboratory Animal Scientist's Association (LASA, India) on "Recent Advances in 3Rs and Laboratory Animal Science", which was jointly organized by Prof. Rana P. Singh, Jawaharlal Nehru University (JNU, Delhi), Dr Vijay Pal Singh, Institute of Genomics & Integrative Biology (CSIR-IGIB, Delhi), and Dr Shikha Yadav, National Institute of Biologicals (NIB, Noida) on November 25-26, 2017 at Delhi, India. LASA, India is a national organization established in 2004 that is dedicated to advancing laboratory animal science by promoting the ethical care and use of laboratory animals in biomedical research, education, and regulatory testing and encouraging scientists to follow the 3Rs (Reduction, Refinement and Replacement). This association has around 660 members, who are veterinarians, scientists, technical experts, students from research and education institutes, as well as from industry.

The organizers of the conference had chosen recent advances in the 3Rs as the theme as it was very strongly felt that at the current stage, it is important to create more awareness as well as a better understanding of the concept of the 3Rs in India to improve animal welfare as well as the quality of research. In India, experiments on animals are strictly regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), which is a statutory body under the Prevention of Cruelty to Animals Act (1960) and promotes the implementation of the 3Rs through the act, rules, guidelines, and further through the CPCSEA nominees in the Institutional Animal Ethics Committees (IAEC) of all institutes in the country that use laboratory animals.

Although awareness about the 3Rs concept is continuously increasing, a level of understanding that could lead to actual implementation is less prevalent. Therefore, it was thought that this conference would provide a platform to Indian scientists for an enriching interaction with experienced international speakers from organizations around the globe like NC3Rs (UK), ECVAM (EC), AAALAC (USA), Jackson Laboratory (USA), Tohuko University, Japan and several more, along with the national experts in the field of laboratory animal science. The event not only increased knowledge and understanding of the recent advances in the 3Rs but also energized all the participants to improve their standards of animal welfare, to implement the 3Rs, and to design much more robust experiments to obtain reliable and reproducible results, thus enabling them to perform high-quality research and regulatory testing.

The conference was preceded by two pre-conference workshops on "Experimental Design" by NC3Rs, UK and "Hygiene in Laboratory Animal Facilities" by Tecniplast, Italy. The delegates attending the hands-on workshop on "Experimental Design" gained a deeper insight on the key elements of experimental design, analysis of the results, and their reporting in international journals as per the ARRIVE guidelines. They also gathered information about the NC3Rs' experimental design resources and were given a live demonstration of the Experimental Design Assistant (EDA), which is freely available on the NC3Rs website for use by scientists around the globe (see below). The workshop on "Hygiene in Animal Facilities" was also of great interest as a good hygiene status is imperative to high quality animal husbandry, which must minimize the introduction and spread of infectious diseases and especially ensure that pathogenic or opportunistic microorganisms are not introduced into specific pathogen-free (SPF) or immune-compromised animals.

The event included participation of renowned keynote speakers, as well as oral presentations and poster sessions held by international and national professionals. Invited speakers and more than 300 participants from all over India and 10 different countries such as USA, UK, Australia, Japan, Korea, Sudan, Sri Lanka, the Netherlands, Singapore, and Denmark came together to share and advance knowledge about recent advances in the 3Rs and laboratory animal science at this event. The participants of the conference included scientists, post graduate students, veterinarians, and technical experts representing various research and educational institutes, universities, industry, and various national and international laboratory animal associations. This scientific gathering will certainly help in the implementation of the 3Rs in research, education, and regulatory testing, which in turn will improve the quality of research as well as animal welfare in India.

As it is not possible to summarize the entire conference, we have made an attempt to summarize the major presentations and discussions that took place at the pre-conference workshops and the conference for wider dissemination of this knowledge.

Disclaimer: This report has been prepared by the authors as a factual summary of the presentations and discussions that took place at the preconference workshops and the conference. The views made are those of the individual speakers and do not necessarily represent the views of the organizers, participants, or of LASA India.

Pre Conference Workshop on Experimental Design

The workshop on Experimental Design was organized by Dr Mark Prescott, Dr Nathalie Percie du Sert, and Dr Viki Hurst from the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs), UK and included presentations, demonstrations, and interactive exercises. The speakers highlighted poor reproducibility and reliability of biomedical research using animals and the factors leading to this, such as poor-quality experimental design, analysis, and reporting. The workshop was planned with the following learning objectives:

- To learn more about current issues with the design, analysis, and reporting of animal experiments
- To refresh the knowledge of the key elements of experimental design and learn to report them in publications
- To give information about the NC3Rs experimental design resources
- Live demonstration of the Experimental Design Assistant (EDA)

Dr **Viki Hurst** (Science Manager – Experimental Design, NC3Rs) focused mainly on the implementation of the ARRIVE Guidelines (https://www.nc3rs.org.uk/arrive-guidelines), which consist of a 20-item checklist summarizing the minimum information necessary to describe *in vivo* studies comprehensively and transparently. These guidelines, which have been endorsed by over 1,000 journals internationally, help to ensure reproducibility and maximize the output of animal research, and also to avoid unnecessary animal use.

Dr **Nathalie Percie du Sert** (Head of Experimental Design and Reporting, NC3Rs) demonstrated the Experimental Design Assistant (EDA; https://eda.nc3rs.org.uk), which is a web application with a supporting website that helps researchers design robust animal experiments by increasing the transparency of the experimental plan and providing feedback to improve it. The key features of the EDA are:

- A computer-aided design tool, to develop a visual representation of your experimental plan;
- Critical feedback on your experimental plan, using computer-based logical reasoning;
- Suggestions for appropriate statistical analysis;
- Sample size calculators;
- Generation of a randomized sequence for allocation of animals to treatment groups;
- Support for allocation concealment and blinding.

The CAMARADES-NC3Rs Systematic Review Facility (SyRF; http://syrf.org.uk), which is a free-to-use online platform for researchers to perform systematic review and meta-analysis of animal studies, was also demonstrated. The platform provides guidance for the entire process and allows some of the tasks to be automated, using data mining and machine learning.

The workshop was highly appreciated by the participants, who felt much more confident in performing reliable and reproducible research by designing more robust experiments. Participants also felt that knowing about the ARRIVE guidelines would enable them to report their experiments appropriately so that the research methods and findings can be fully understood.

Preconference Workshop on Hygiene in Laboratory Animal Facilities

"Happy and healthy animals make good science", so a good hygiene status is imperative to high quality husbandry. It has been noticed that in the Indian scenario, the importance of proper cage sanitizing is often underestimated, even though it has a critical impact on both animal health and welfare. The workshop on Hygiene in Laboratory Animal Facilities was organized by Tecniplast, Italy in association with Samitek Instruments, India.

It is a pre-requisite to minimize the introduction and spread of infectious diseases to ensure that pathogenic or opportunistic microorganisms are not introduced, especially into specific pathogen-free or immune-compromised animals and that any experimental biological hazards are completely destroyed before cages are reused to house other animals.

The speakers of the workshop were Mr **Franco Mondini** (Techniplast, Italy); Dr **Amol G. Ganla** (Medivue Technologies Pvt. Ltd.), Mr **Riccardo Saggin** (IWT,Italy), Mr **Tomas Tucek** (BMT – MMM group), and Dr **Rajesh Anand** (Samitek Instruments), who are experts in the areas of disinfection, sterilization, and washing solutions. The experts talked on critical topics like:

- Is your cage clean? The need for a validated cage sanitizing process;
- Damages on cage surfaces and corrosion – causes and prevention;
- How to decontaminate individually ventilated cages (IVC) and laminar air flow equipment by using Decon Technology;
- Sterilization: Basics for animal facilities.

This workshop was very useful as participants learnt that appropriate washing, sanitization, and decontamination of cages is critical for animal welfare as well as for keeping animals healthy for research, which in turn is a prerequisite for obtaining good results.

Plenary Talk – Capability Maturity Model for Culture of Care

The plenary talk was delivered by Dr **Suresh Poosala** (Senior Group Director, Bristol-Myers Squibb, USA) on "Capability Maturity Model for Culture of Care". He explained that this model has been developed with the aim to have highly competent people taking care of laboratory animals by instilling a culture of care, which eventually leads to meaningful research and enhances the value of what can be done to address the various needs of mankind. He discussed the model, its evolution, its content, and finally what it takes to reach the altars of good care. He said the model provides tools for the participants to measure the culture of care they have at their sites and to make suitable adjustments to their programs.

To achieve a culture of care in their respective organizations, leaders must implement several methods. These methods range from enhancing the infrastructure to training of the staff and all the related aspects in between and around these. While they do this, there is a good chance that such leaders become compla-

cent at some point and believe that they have achieved what they wanted for their organization. Dr Poosala said that it is simply impossible to arrive at this level of satisfaction or complacency if proper measures are put in place to regularly assess and challenge these assumptions. Best care is a dynamic, moving target and cannot be measured in simple terms.

In the presentation, an attempt was made to design a model that originates from successful international management programs and integrate this into local facilities. This was one such first attempt in the field of laboratory animal science and will certainly help the participants to implement a culture of care in their organizations.

Keynote Lecture – Scientific Importance of the 3Rs: An Introduction to the NC3Rs and its Education and Training Resources

Dr **Mark Prescott** (Director of Policy and Outreach, NC3Rs) gave an introduction to the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs), UK, which is an independent, scientific organization established by the UK government to lead the discovery and application of new technologies and approaches that avoid or minimize the use of animals in research and that improve animal welfare where their use is necessary. The Centre uses a range of strategies to improve and advance science through application of the 3Rs, including funding research, early career development, open innovation in universities, companies and SMEs, and leading cross-sector data-sharing initiatives to provide an evidence base for changes in policy, practice, and regulations. An important part of its work is to provide researchers, veterinarians, animal technicians and others with the practical tools and information to put the 3Rs into practice, which is done principally through workshops and networking events, web-based resources, and publications.

Through this presentation, Dr Prescott highlighted the NC3Rs' high-quality education and training resources on topics such as experimental design and reporting for *in vivo* studies, humane conduct of scientific procedures on animals (e.g., administration of substances, blood sampling, anesthesia), and methods for welfare assessment. These resources are used internationally, which has been supported by language translations in some cases. Some of the education and training resources from the NC3Rs (e.g., grimace scale posters) were also shared with the participants at the conference to help them put the 3Rs into practice.

Dr Prescott further emphasized how a 3Rs approach can help scientists to improve the internal validity of their experiments, data quality, and also maximize the information gained from their *in vivo* research, thus facilitating scientific discoveries and medical advances.

Replacement

Replacement refers to the use of alternative methods that avoid or replace the use of animals. This includes both absolute replacements, i.e., replacing animals with inanimate systems, such as *in vitro* systems of human origin and computational methods, and relative replacements, i.e., replacing more sentient animals such as vertebrates, with animals that scientific evidence indicates to have a lower potential for pain perception, such as *Drosophila*, nematode worms, and social amoebae, or use of primary cells (and tissues) taken from animals killed solely for this purpose. There are also commercially available primary cells of human origin, which are interesting for research as they enable the understanding of human biology and underpinning disease mechanisms in humans. However, human primary cells are expensive and have a short lifetime in the laboratory, which is why cell lines originating from human cancerogenic tissues that are easily cultured in the laboratory (e.g., HepG2, HepRG) are often used to confirm human relevance. More recently, pluripotent stem cells taken from human tissues, such as skin, have been efficiently differentiated into almost any human cell type and cultured also in large scale for high throughput testing, which is providing human relevant and metabolically competent *in vitro* models. Such differentiated pluripotent stem cells are also used in organ-on-a-chip devices, which are now provided by many biotechnology companies. They include 3D cultured cells in flow-through systems, sometimes also with several different compartments corresponding to different human organs.

To date, replacement alternatives have been established and validated especially in studies where effects involve only a single cell or tissue type. However, it is much more difficult to establish replacement strategies for processes that involve more dynamic and complex biological interactions. Here integration of both *in vitro* and computational methods must be applied. It should be understood that animal use is not our right, but a privilege that requires close adherence to a clear and defined code of ethics to ensure that all animals are treated as humanely and judiciously as possible. Moreover, for research projects involving animals, we must now actively look for and consider the use of potential alternatives, if they have been shown to result in sound scientific conclusions.

Dr **A. B. Pant** (CSIR-Indian Institute of Toxicology Research, Lucknow-India) informed the participants about the development of alternatives to laboratory animals in India. He said that CSIR-IITR had initiated a flagship project in 2002 with the primary aim of developing and validating *in vitro* and lower invertebrate as well as plant-based alternative models for biomedical research. In the first phase, they established and validated over 50 globally accepted OECD-approved cell based and alternative models for basal and organ specific toxicity, genotoxicity, eco-toxicity, and pesticide residue analysis. These internationally accepted models are extensively used in the regulatory studies referred to the CSIR-IITR. In the second stage, they undertook to develop and validate newer and more sensitive human-specific *in vitro* and alternative models of neurotoxicity, developmental neurotoxicity, cytotoxicity, pyrogenicity, phototoxicity, hepatotoxicity, immune-toxicity, genotoxicity, oral toxicity, and so forth. The development of pluripotent human cord blood-derived hematopoietic and mesenchymal cells gave them new tools to improve and develop *in vitro* 2D and 3D models that better mimic human phys-

iology. Many of these models have already completed intra- and interlaboratory validation and the data is ready to be sent for regulatory inspection. Dr Pant also informed that attempts are being made to develop country-specific "National SOPs for the Use of Experimental Animals in Medical Education and Research".

Dr **Elisabet Berggren**, Deputy Head of the Chemicals Safety & Alternative Methods Unit at the JRC (European Commission), which hosts the European Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) said that EURL ECVAM is mandated by EU law to protect animals used for scientific purposes and to reinforce the 3Rs. At EURL ECVAM, innovative non-animal test methods, primarily for the regulatory safety assessment of chemicals, are developed, evaluated, harmonized, and promoted. It is now generally accepted that no more animal testing is required for topical toxicity testing and major resources are now being employed to support the development of alternative methods in an integrated approach to testing and assessment (IATA) to tackle systemic toxicity. It is believed that with better science, applying human-relevant methods, a more cost- and time-efficient assessment of chemicals, providing a higher level of safety for workers and consumers and at the same time safeguarding the protection of animal welfare, can be achieved. EURL ECVAM provides support to a broad range of policy areas, including industrial and household chemicals, cosmetics, food, plant protection products, endocrine disrupters, and chemical mixtures. EURL ECVAM also works on strengthening knowledge exchange to progress the 3Rs between different areas of expertise and develop education on how to apply new scientific solutions to protect human health and the environment.

Mr **Nick Jukes**, coordinator of InterNICHE (International Network for Humane Education, connected by video conference) informed the participants on the best practice and alternatives that can be used in education and training.

Reduction

Reduction of animal experiments is not only about obtaining the best quality and most precise information with the smallest possible number of animals, but it is also about well-designed and well-conducted experiments that deliver reliable and reproducible results.

Dr **A. Sankaranarayanan** (Vivo Bio Tech Ltd Hyderabad) discussed the "Development and Use of Humanized Animal Models in Drug Development". Humanized mice are immunodeficient mice engrafted with human tissues or cells (HSCs, PBMCs). The laboratory mouse is the most widely used animal model in biomedical research. However, mice and men differ from each other in their complex biological processes, which limit the utility of mice in experimental medicine and drug development. To address this translational issue, "humanized mice" have been developed that are being used in basic and applied biomedical research and drug development. The humanization of mice is carried out by engrafting mice with human cells or tissues, or by genetically modifying them by introduction of human transgenes. With the ability to develop increasingly high-level immune deficient mice, grafting of human cells and tissue in mice has become more efficient. Similarly, generations of genetically humanized mice have undergone extensive improvements. Having started with techniques like injection of human cDNAs into fertilized eggs, sophisticated genome editing technology like CRISPR/Cas9 is currently used to generate these models. These models find application in wide areas of experimental medicine and drug research. They are used in compound efficacy and safety testing, drug metabolism, investigations on immune system functions and related research.

Dr **Nathalie Percie du Sert** (NC3Rs, Head of Experimental Design and Reporting) informed the participants about the "NC3Rs resources to improve the experimental design, analysis and reporting of animal studies". Dr Percie du Sert pointed out serious issues regarding the reproducibility of animal studies and their translation to humans. Poor study design, investigator bias, and incomplete reporting have been identified as major contributing factors for what some commentators have referred to as a reproducibility crisis. The NC3Rs has been working in this area over the last ten years and initiated the development of two key resources to support researchers and improve the design, analysis, and reporting of *in vivo* experiments.

The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines consist of a 20-item checklist, which summarizes the minimum information necessary to describe an experimental study in animals in a comprehensive and transparent manner. The guidelines cover the main aspects of a scientific publication and make recommendations on the reporting of the study design, experimental procedures, animal characteristics, housing and husbandry, and statistical analysis.

The Experimental Design Assistant (EDA; https://eda.nc3rs.org.uk) is a secure app that uses computer-based logical reasoning to guide researchers through the design of their animal experiments, highlighting issues that could compromise the rigor, and ultimately the reproducibility, of the research. The EDA provides tools to blind the experiment, randomize animals to groups, and also provides power calculators for determination of the necessary sample size to achieve statistically relevant results.

The CAMARADES-NC3Rs Systematic Review Facility (SyRF; http://syrf.org.uk) is a free-to-use online platform for researchers to perform systematic review and meta-analysis of animal studies. The platform provides guidance on the entire process and allows some of the tasks to be automated, using data mining and machine learning.

The objective of these resources is to maximize the output of research using animals and wide dissemination in India would certainly benefit the Indian scientists.

Dr **Anurag Agrawal** (Director, CSIR-Institute of Genomics and Integrated Biology, Delhi) gave a detailed talk on "Statistical & Methodological Issues for Biologists" wherein he discussed statistical fundamentals including different data types, factors to be kept in mind while deciding the sample size, and factors which add variation in animal experiments. He also explained how to select the appropriate statistical test depending on the types of variables, type of research question, and the data structure.

Grace E. Berryhill (The Jackson Laboratory, USA) discussed designing and successfully carrying out robust mouse studies by

- Selecting appropriate mouse models and controls, as reproducibility depends on a thorough understanding and characterization of the selected mouse model(s);
- Suitability of phenotype readout- choosing quantifiable assays and meaningful readouts;
- Plan for sufficient mouse sample size to adequately answer the research question.

Refinement

Animals used in research are inevitably exposed to stressors, both during husbandry and during experimental procedures. However, all efforts should be made to refine the experiments to make sure that animals suffer as little as possible by providing better housing and husbandry, minimizing pain and suffering by early and effective recognition of pain and use of appropriate analgesia and anesthesia, and implementing humane endpoints. Researchers, veterinarians, and the animal care staff should act as a team and together play an important role in implementation of enrichment, as providing appropriate enrichment promotes normal behavior and behavioral diversity and increases the ability of the animals to cope with challenges in a more normal way while reducing the frequency of abnormal behavior.

Dr **Noriyuki Kasai** (Vice President and Secretary General of ALFAS and Professor Emeritus and Guest Professor at Tohoku University Center for Laboratory Animal Research, Japan) discussed about "Environmental Enrichment for Laboratory Animals, especially the practice of enrichment for monkeys and pigs in Tohoku University", Japan. He emphasized that science has an ethical responsibility to house animals according to their species-specific needs, and that responsibility invokes the concept of behavioral and environmental enrichment. It is important to enhance the environment of confined animals in order to encourage natural behaviors and improve their quality of life. Enrichment not only has the potential to improve the animals' welfare during husbandry but could also help to refine the experimental procedures.

Dr **James Swearengen** (Global Director, AAALAC International, USA) enlightened the participants about "Enhancing the Three Rs through Post-approval Monitoring". In the *Guide for the Care and Use of Laboratory Animals (Guide)*, NRC 2011, the continuing oversight of animal activities by the Institutional Animal Care and Use Committee (IACUC) is referred to as post-approval monitoring (PAM). *The Guide* describes a wide variety of monitoring strategies, from very simple to very intricate, and recommends that they be tailored to the complexity of the research program. The Three Rs are commonly considered during the experimental design phase of an animal research proposal, but a key component of PAM is to ensure that the animal procedures being performed conform to the procedures described in the IACUC approved protocol. While PAM helps to ensure the well-being of animals as IACUC approved activities are occurring, *the Guide* also suggests that it offers the opportunity to refine research procedures. This provides an opportunity to use PAM to advance the ongoing implementation of the 3Rs. Developing a PAM program that actively strives to identify opportunities to apply the Three Rs will not only enhance animal welfare, but can also support better science. Dr Swearengen emphasized that in all cases, a PAM program should be a collegial and communication-intensive partnership between those performing the PAM and the research support staff and investigators, with everyone involved understanding the benefits it provides to both the animals and the science.

In the Indian scenario, CPCSEA as well as IAECs constituted by them consider the 3Rs while providing ethical approvals for research protocols and they periodically review the progress under these protocols until completion of the projects. However, the information about PAM was very useful as there is a lack of implementation of PAM at intricate levels to ensure that the animal procedures being performed by researchers actually conform to the procedures described in the IAEC approved protocols. This in turn would help to enhance the 3Rs and support better science as well as improved animal welfare.

Kathryn Bayne, (Chief Executive Officer, AAALAC International) delivered a talk on "Promoting the 3Rs though the Appropriate Use of Environmental Enrichment". The importance of species-appropriate environmental enrichment to promote the welfare of research animals is an accepted ethical principle, but its impact on the research data initially received little attention. However, this has now changed as enrichment provided as one element of routine husbandry has moved from larger research animals, such as nonhuman primates, to small research animals, such as rodents and fish. Concern has been expressed that enrichment negatively impacts both experimental validity and reproducibility. However, Dr Bayne clarified that when a concise definition of enrichment is used, with a sound understanding of the biology and behavior of the animal as well as the research constraints, it becomes clear that the welfare of research animals can be enhanced through environmental enrichment without compromising their purpose. Indeed, it has been shown that the converse is true: the provision of suitable enrichment enhances the well-being of the animal, thereby refining the animal model and improving the research data. Dr Bayne further emphasized that both the validity and reproducibility of the research are enhanced when proper consideration is given to the research animal's living environment and the animal's opportunities to express species-typical behaviors, thus meeting the tenets of the 3Rs – in particular those of Refinement and Reduction.

Dr **Beena Pillai**, CSIR-IGIB, Delhi talked about a novel refined method to develop ataxia in mice. MicroRNAs are small regulatory RNAs that bind to complementary target sites in untranslated regions and repress the translation of messenger RNAs. They identified down regulation of the microRNA miR-29 as a common event in patients and animal models of several neurodegenerative disorders including Alzheimers's disease, Huntington's disease and spinocerebellar ataxias. They have used a combination of locked nucleic acid based antagomiRs with a neurotropic cell penetrating peptide to knock down miR29 in the mouse brain transiently and have established that a transient loss of the microRNA over 4 days was sufficient to cause extensive cell death and ataxia in the mouse. Using this novel refined method, they

were able to establish an alternative protocol to the technically challenging, tedious, and painful procedure of stereotactic injection used in these disease models.

Dr **Patricia V. Turner** (Dept of Pathobiology, University of Guelph, Canada) talked about "Pain recognition, assessment and mitigation in laboratory animals". Despite significant research and knowledge in the area of pain recognition in research animals, there are several challenges to adequately mitigate pain and distress that arise from experimental design issues, i.e., the ability to adequately see and monitor animals, distinctive animal genetics and their effect on response to analgesia therapy, access to specific therapeutic agents, practicalities of using certain techniques, as well as availability of robust training programs for personnel. To ensure the best outcomes for research animals, it is important to have robust pain recognition and assessment methods in place and to use a multimodal approach to pain management to minimize potential side effects. Dr Turner discussed updates in laboratory animal analgesia management including the confounding effects of pain on research, developments in pain monitoring and recognition, and considerations for treatment protocols. It was emphasized that provision of appropriate analgesia for research animals is a critical consideration for the veterinary care and research teams, as well as for the animal ethics committee that reviews the project to ensure good animal welfare.

Edwin N. Spoelstra, (Sales Director Instech Laboratories Inc., Europe and India) delivered a talk on "Improving welfare of catheterized mice and rats". Catheterized mice and rats are commonly used for blood sampling and intravenous infusion. Blood sampling and chronic intravenous infusion play an important role in glucose clamp, toxicology, drug metabolism and pharmacokinetics and safety pharmacology studies. An indwelling blood catheter is exteriorized and coupled to an external port (so called vascular access button) and sampling tubing through a metal spring connected to a swivel system. This enables the animal to move freely while blood is collected or test compound infused (combination of infusion and blood sampling). Since the introduction of the external port, several product modifications have improved animal welfare and the most important improvements were presented by Mr Spoelstra to enable the researchers to adopt these to refine their experiments.

Nicholas Tan (Regional Manager STERIS Life Sciences) spoke about "Animal facility bio-decontamination using VHP". The vaporized hydrogen peroxide (VHP) decontamination system is a self-contained hydrogen peroxide vapor generator designed for decontamination of sealed enclosures (isolators, work stations, aseptic filling lines, pass-box, Hepa Filters) and rooms in research, biological safety, HVAC, and production applications. VHP is registered with the U.S. Environmental Protection Agency as a sterilant and is sporicidal, leaves no residue, has fast cycle times, reduced downtime for facilities, is compatible with most materials, and is safe. Hydrogen peroxide vapor processes provide a wide range of applications in both facility commissioning and prophylactic bioburden management, which in turn would prevent infections in animals and improve animal welfare.

Franco Mondini (International Sales Director, Tecniplast) introduced the participants to the new concept of "Animal welfare monitoring in the *home cage*: Introducing the DVC® (Digital Ventilated Cage)". Laboratory procedures and conditions can exert influence on an animals' physiology and behaviors that is difficult to control and that can ultimately impact research outcomes. To reduce such influences, the concept of the home cage and the possibility of non-invasive, 24x7 animal welfare and activity monitoring is gaining ground. Automated home cage monitoring is based on a form of automation and allows the opportunity for more effective welfare outcomes. Automated home cage monitoring complements behavioral testing, improves the animal welfare components as well as study interpretations, and can also add to operational resource efficiencies, decrease waste, and decrease study interference while maintaining animal welfare. Also, the health and safety of the individuals handling animals and/or the equipment used in association with experimental models is ensured through the enforcement and proper management of the hygiene routines throughout the animal facility.

Biosafety risks with laboratory animals

The use of animals in scientific research has enabled major advances to be made in biomedical research in the areas of surgery, physiology, toxicology, pharmacology, infectious diseases, and other disciplines. The surge in basic and applied biomedical research was accompanied by the increasing use of animal models. However, working with laboratory animals presents significant biosafety risks.

Dr **Cecelia V. Williams** (Sandia National laboratories, USA) discussed about "Biorisk Management in Laboratory Animal Use" and informed the participants that biorisk management is a system or process to control safety and security risks associated with the use, handling, storage, and disposal of biological agents and toxins in laboratories and research facilities. The "AMP" (Assessment, Mitigation, and Performance) model is a simple but powerful approach for managing biorisks. In addition to working safely and securely with laboratory animals, we must work humanely when conducting animal research to ensure ethical treatment of animals. The principles of the 3Rs were developed to provide a framework for performing more humane animal research and Dr Williams emphasized how the 3Rs tenets are anticipated outcomes of the implementation of an effective biorisk management system.

Dr **Lars Friis Mikkelsen** (CEO, Ellegaard Göttingen Minipigs A/S, Denmark) discussed the prospects of using Göttingen minipigs in biomedical research. In recent years, minipigs have emerged as an alternative to dogs and non-human primates in both preclinical toxicology and safety testing plus as a non-rodent animal model for pharmacological research within several disease and therapeutic areas like, e.g., diabetes and obesity, cardiovascular diseases, gastrointestinal diseases, skin diseases, ocular conditions plus reproductive toxicology, and juvenile studies. The translational value of the Göttingen minipig is mainly due to similar anatomical and physiological characteristics and important genetic similarities, which makes the Göttingen minipigs the most characterized, validated, and globally most used minipigs in

biomedical research. Further, it was emphasized that the microbiologically defined Göttingen minipigs are bred in barrier facilities and subjected to strict genetic breeding management procedures to minimize in-breeding and maintain the genetic integrity of the population, thus providing an animal model with a high standard of health, welfare, and quality.

"Ask us anything" session

Although the participants had opportunities to interact with the invited speakers and experts after their individual presentations and during the breaks, on the final day of the conference a special "Ask Us Anything" session was organized in which Dr Kathryn Bayne (CEO, AAALAC International, USA), Dr D.S Upadhaya (Scientist, Central Drug Research Institute, Lucknow, India), Dr S.G Ramachandra (Principle Scientist, Indian Institute of Science, Bangalore, India), Dr Sankaranarayanan (President, Vivo Bio Tech Ltd Hyderabad), and Dr Suresh Pothani (Director, NCLAS,NIN,Hyderabad, India) were kind enough to participate.

In this session, the participants had ample opportunities to interact directly with these stalwarts in the field of laboratory animal science and ask questions, share their ideas, viewpoints, and experiences. There were also discussions on how the implementation of the 3Rs can be spearheaded in India and the experts helped the participants resolve the challenges they may face with regard to implementation at their workplace.

In India, CPCSEA enforces the 3Rs through the Prevention of Cruelty to Animals Act (1960), Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, which were further amended in 2001 and 2006, and its strict guidelines. CPCSEA also recognizes the 4th R, which involves the concept of Rehabilitation and Reuse of animals of higher phylogenetic order such as dogs and non-human primates. This has evolved as an official policy of the CPCSEA since 2004 as mandated by law, i.e., Rule 9 (c) of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, which states that "animals intended for the performance of experiments are properly looked after both before and after experiments."

However, it was strongly felt by all participants that like the UK, Europe, Japan, and other countries, the Indian government too should initiate the process to establish an organization that would work exclusively to promote awareness for the 3Rs, by disseminating knowledge through training and helping Indian scientists to incorporate the 3Rs in their experimental designs to obtain scientifically sound results, avoid any unnecessary duplication of animal based experiments, and also to refine their experiments to minimize pain and suffering in the experimental animals. This would enable scientists in India to forge a way ahead to promote humane, responsible animal care and use in experiments, thus enhancing the quality of research, teaching, and testing.

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