Advancements of 3Rs in Biomedical Research, Laboratory Animal Science and Welfare: International Perspectives

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Abstract:

The article summarises the outcomes of an international conference of Laboratory Animal Scientists' Association (LASA, India) on "Advancements of 3Rs in Biomedical Research, Laboratory Animal Science and Welfare: International Perspectives" which was organised by the Conference Committee and Organising Secretary, Dr. R K Shakthi Devan, Syngene International Ltd, Bangalore; Secretary, Laboratory Animal Scientists' Association; and Indian College of Laboratory Animal Medicine at National Science Seminar Complex, JN Tata Auditorium, Indian Institute of Science, Bangalore on 7 and 8 November 2023 and preconference workshops also held on 6 November 2023. LASA India is a national organization dedicated to advancing laboratory animal science by promoting the ethical care and use of laboratory animals. LASA has more than 1000 life members from biomedical professionals including Veterinarians, Scientists, Academicians, Researchers, Technical experts from industry, state and central institutions in the field of laboratory animal science. The organisers felt a need for the promotion of 3R's (Replacement, Reduction and Refinement) through this international event which was planned meticulously.

Keywords *- 3R's Principles, Specialty Certification, non-clinical studies, Regulatory, Animal Welfare.*

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Introduction

The international conference (LASACON 2023) has organized four pre-conference workshops named as '*Key Concepts and Resources for Design and Reporting of Animal Experiments by NC3Rs*'; '*Indian College of Laboratory Animal Medicine; Current Status and Path Forward*'; '*Importance of Animal Health and Genetic Quality in your Research'*; and *Training of Animal Care Technicians for Laboratory Animal Support Program*'. The main conference talks were scheduled with 10 sessions in a parallel manner for the benefit of a wider audience. Over 570 registered participants / speakers from India, USA, UK, Sweden, Germany, Denmark, Italy, Japan, South Korea, Thailand, Singapore, Malaysia and Sri Lanka attended this international event. Eminent speakers from Industry, Research Institutions, Academic and Government agencies like the Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Dairying and Animal Husbandry, Government of India addressed the gathering. Similarly, expert participation was by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R's) which is a UK-based scientific organisation dedicated to helping the research community worldwide to identify, develop, and use 3R's technologies and approaches. The event included an inaugural address, keynote talk, scientific and technical presentations by the experts and posters also displayed by the participants in the field of biomedical research.

Contemporary topics were chosen by emphasising the recent advancements of 3R's and enabling the research needs with upright animal welfare. The congregation of biomedical professionals certainly help implement 3R's principles and advancements in the laboratory animal science arena. The International Conference was inaugurated by the Chief Guest **Dr. SK Dutta**, Joint Commissioner (AH) & Member Secretary (CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, enlightened the statutory requirements and functioning of laboratory animal establishments in India. The Guest of Honor **Dr. R Gopinath** IFS, Deputy Secretary, All India Institute of Medical Sciences (AIIMS), spoke about laboratory animal science and its importance in research by upholding the welfare. The Guest of Honor, **Dr. Mahesh Bhalgat**, Chief Operating Officer, Syngene, reflected on the role of laboratory animals in biomedical research and provided insights on global scenario from the industry standpoint. The events were conducted with several scientific and technical sessions and we have highlighted the summary discussions of the conference as part of dissemination of the information.

Preconference Workshop - 1

Key Concepts and Resources for the Design and Reporting of Animal Experiments.

Dr. Jessica Eddy and **Dr Nicola Foster** discussed the three-hour workshop organised by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK. They discussed issues in the way animal research is currently designed, conducted and reported in the scientific literature. The speakers covered key concepts in the design of animal experiments such as sample size determination, randomisation and masking (blinding) and presented tools and resources available to support researchers. This includes the Experimental Design Assistant (EDA), an online expert system to guide researchers through the design of animal experiments, and the ARRIVE Guidelines, a checklist of recommendations to improve the reporting mainly study planning, study conduct, writing, and review of research involving animals in biomedical science. In addition, RIVER recommendations were also covered which helps researchers design robust *in vitro* experiments.

Preconference Workshop - 2

Indian College of Laboratory Animal Medicine (ICLAM)

The workshop session covered a wide range of topics about laboratory animal medicine by the experts and Diplomates from different member colleges such as ACLAM, ECLAM, ICLAM, and JCLAM.

Training Opportunities and Routes to Examination, the ECLAM Experience'

Dr Patricia Hedenqvist, President of IACLAM deliberated that the ECLAM was founded in the year 2000 and has grown to a total number of 115 Diplomates, of which 73 are active. With only four formal residency programs, most Diplomates are trained by an alternative to the residency route. The minimum duration and requirements of training are the same regardless of route, whereas the alternative route used to be longer. In 2019, there was a decision to increase the membership number by offering American and Asian LAM Diplomates to be recognised as Diplomates of ECLAM, if they were employed in Europe. Further, the possibility of undergoing the certifying exam without previous residency training was introduced to internationally recognised LAM specialists. The publication requirements for residents in training were also reduced, in harmonisation with other LAM colleges. ECLAM abided to follow rules by an umbrella organisation for all European veterinary colleges (European Board of Veterinary Specialisation) and can only change its rules within that framework. During the workshop, the ECLAM experience was compared with that of the other LAM member Colleges. She has emphasised the importance of board certification and its impacts on animal welfare, quality of science, and benefit to humans and animals. The actions and plans embrace the international mentorship program, workshops and round tables, develop best practices and promotion of laboratory animal veterinarians through the specialty certification program.

Updates from the American College of Laboratory Animal Medicine (ACLAM)

Dr Anjela King-Herbert, Past President, ACLAM provided updates on ACLAM especially the roles of Diplomates such as animal resource management, providing adequate veterinary care, advising scientists, assistance in achieving regulatory compliance, research, training and education. She had explained the current status and program requirements including the strategic plans including the vision and mission, short- and long-term goals, objectives, and action plans including the task force. A strategic plan enhances and broadens the ACLAM activities viz recertification program, role delineation document, training program recognition, continuing education and publications, establishment of foundation, and supporting research in laboratory animal science and medicine. The strategic plans with specific assumptions and performance measurements that contain digital technology, advocacy within the American Veterinary Medical Association (AVMA) through American Society for Laboratory Animal Practitioners (ASLAP), advocate for diversity, equity and inclusion, ensure the diplomates are valued as critical assets in biomedical research. She highlighted about the residency program and several other activities undertaken by different committees of ACLAM board.

Contribution of Laboratory Animal Medicine Specialists

Dr Tsutomu Miki Kurosawa, Past President of IACLAM and JCLAM enlightened about the systems followed at JCLAM and contributions of other member colleges through laboratory animal medicine specialisation. He has emphasised about the importance of animal experimentation, laboratory animal welfare including companion animals and the role of veterinarians for their contributions in the

academic, industry and policy-making related to laboratory animal medicine. He cited about WOAH (OIE) Animal Welfare Terrestrial Animal Health Code (2023), ILAR Guide, CIOMS-ICLAS guiding principles, ISO 10993-2, IACUC constitution and its sections associated with animal welfare. He stressed that international harmonisation and standardisation is important in the scientific field and further elucidated about Prof Ben Cohen who was one out of five founders of the American Association of Laboratory Animal Science (AALAS) and instrumental in establishing Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and American College of Laboratory Animal Medicine (ACLAM). He urged the animal care and refinement of the 3Rs aspects through specialists of laboratory animal science and medicine.

ACLAM Examination Process

Dr Jennifer Lofgren, Global Head of Animal Welfare Compliance at Novartis, USA described the ACLAM examination including the application process, exam fees, exam format, and opportunities for exam preparation such as the mock exam and Camp ACLAM. She explained about the importance of examination preparation and pass rates of ACLAM. Further, stated about the training program route as well as experience route apart from the requirements to take the examination including the pattern being followed and conduct of the examination. The role delineation document (RDD) with primary, secondary, and tertiary species and other reference resources were also discussed including the publications. She also mentioned the exam committee and the contracted services to develop, administer, and score the ACLAM certification exam. The scoring of the exam and reporting of results occurs after the consultation with the exam committee, the exam results are presented to the ACLAM Board of Directors at their meeting at the AVMA National Convention. In addition, she has informed about the study group and other resources available for the candidates which helps them while preparing for the examination.

ICLAM Current Status and Path Forward

Dr Shakthi Devan, Secretary ICLAM, briefed about the establishment of ICLAM and, the registration process under the Tamil Nadu Societies Registration Act 1975 (Tamil Nadu Act 27 of 1975) and other administrative steps involved in fulfilling the membership requirement of International Association of Colleges of Laboratory Animal Medicine (IACLAM) to be part of the member colleges. He also acknowledged that several Diplomates from IACLAM supported the establishment of ICLAM in India. The first batch of candidates who have cleared their eligibility and appeared for the board examination and awarded Diplomate status at this event. He further added that the ICLAM continues to work with the member colleges based on the strategic plans and strengthen the specialty program in India.

Registration Procedure, Training, and Experience Requirements of a Board Examination of ICLAM

Dr Arvind Ingle, President of ICLAM, deliberated that the ICLAM is offering the certification of veterinarians in the field of laboratory animal medicine in India. Veterinarians, who are registered with the Veterinary Council of India (VCI) and are working in laboratory animal science, can apply for the specialty training and pass the examination successfully to be entitled to the award of Diplomate, ICLAM, or DICLAM. It would be appropriate to understand the procedure of registration, training, and experience requirements by the prospective veterinarians. In the case of a graduate veterinarian (B.V.Sc & AH), at least 06 years of service in the field of laboratory animal care, breeding, management, and/ or experimentation is a must whereas a

postgraduate (M.V.Sc or Ph.D.), can apply after at least 04 years of service as above. Besides, a veterinarian also needs to be a first author of a minimum two original articles and one corresponding author to demonstrate the application of scientific methods on topics that are relevant to laboratory animal medicine and science. The articles must have been published or accepted for publication in at least a NAAS-rated science journal at the time of application for eligibility to undertake a board examination. Once a veterinarian qualifies for all the above requirements, he/she has to find a supervisor for the training to complete at least 200 hours of didactic training. During the process of one-year compulsory training, the trainees are expected to cover the experience of primary and secondary species. Trainees are also required to submit the monthly/quarterly report to the Registrar, ICLAM through their supervisors. After completion of the one-year compulsory training, the trainees are eligible to take up the examination. Examination will necessarily cover the biology of laboratory animals; laboratory animal resource management; clinical laboratory animal medicine including surgery and post-operative care; laboratory animal pathology; prevention and health monitoring programs; research support and animal experimentation by adopting 3R's principles; regulatory requirements, law, and animal welfare. The candidates need to take up two papers and must pass these two papers with at least 60% marks in each paper to clear the examination before awarding the title of DICLAM which are conferred in the annual meetings in person.

Overview of US Laboratory Animal Residency Requirements & Programs

Dr David Moore, a veteran Diplomate of ACLAM provided insights about the residency programs in US and training program standards. He has deliberated about the evolved program of ACLAM over the years and structured residency training offered in the US. He had elucidated by assessing the ACLAM model for laboratory animal training as a template for developing a plan for training program requirements by ICLAM. He explained the history of ACLAM establishment and progress (1957-1997) to achieve the program standards over 40 years and improve laboratory animal medicine. The Role Delineation Document (RDD) provides a standardizing template for designing the ACLAM-recognised training programs and it aids in formulating continuing education for diplomates with the prescribed domains to attain the knowledge for appearing the board examination. The RDD comprises of prevent, diagnosing, control, treat disease, minimise pain and distress; providing research support information and services; developing and managing animal husbandry programs; executing institutional animal care and use committee veterinary responsibilities; designing and operating laboratory animal facilities; provide consultation governing appropriate care and use of laboratory animals; educate scientific, animal care, and ancillary staff; Collaborate on the selection and development of animal models; design and conduct of research. Evaluation of the program at periodic intervals is important to assess the effectiveness of the training and implement the programmatic changes, as appropriate. He also explained the categories of organisations with ACLAM recognised training programs followed by the federal government, health science institutes, and public and private universities in the US. He has provided a comparison of features of the various ACLAM-recognised training programs including the requirement for completion of concurrent advanced degree; length of the program and veterinary internship and residency matching program. An overview of the ACLAM program helped visualising the program standards by the audience.

Preconference Workshop - 3

Planning and Commissioning of High-Security Animal Bio-containment Laboratories (ABSL-3)

Dr Harinarayana Rao, Consultant had elaborated on the ABSL-3 prerequisites and risk group descriptions. He has cited risk-based enhancements of system from the general lab to the highest levels of containment. The additional requirements of facilities include personal shower out, double PPEs with powered air-purifying respirators for personnel, provision of exhaust filters and effluent decontamination system. The main purposes are isolation of pathogens for identification and culture; and animal challenge studies for vaccine or drug efficacy testing. He has explained about biosafety and biosecurity by protecting the researchers from accidental exposure as well as to protect community. The containment concept includes primary containment (individually ventilated cages), and secondary containment (Biosafety cabinet) which are mandatory as part of the infrastructure apart from isocages, isolators are required depending upon the types of research. He has touched upon the BSL-3 facility planning, approval requirements, commissioning and functional for the research use. The ventilation and unidirectional airflow pattern, and pressure gradient monitoring is paramount within the facility. The materials must be decontaminated, and the effluent water treatment system is necessary. He has reiterated that the operations of BSL-3 are stringent by adhering to the regulatory requirements including the biomedical waste disposal process following the guideline.

Solutions for Biocontainment

Mr. Carlo Demalde, Tecniplast, Italy, delivered a talk about the biocontainment aspect in the animal facility by installing the equipment. The biocontainment is unique and requires adequate planning and execution of equipment for high-quality animal maintenance with clean status. Several biocontainment methods are available with custom-made infrastructure depending upon the facility's needs. The Isocage is an advanced system with a negative pressure option, a screwed plenum to avoid accidental disassembling and a control cage is available for monitoring. The decon rack is another advanced system that allows for autoclaving of cages. The Isocage Biosafety Station working concept was explained which is autoclavable and the transfer chamber is available apart from a dunk tank provision, dirty and clean cage operations are possible as per the protocol. Further, explained how the contamination can be minimised by using the right kind of equipment. He has briefed about the facility design requirement for the BSL-3 facility and the usage of these caging systems for better containment for conducting laboratory animal research.

Importance of Animal Health and Genetic Quality in Your Research

Karine A. Lux, Associate Director, Operational and Quality, The Jackson Laboratory, USA. Pronounced that the cornerstone of ethically and scientifically sound research lies in the rigorous upkeep of animal health and genetic quality, especially concerning laboratory mice, often the linchpins of biomedical exploration. This session delves into an interdisciplinary framework of roles and responsibilities, emphasizing comprehensive welfare protocols, regulatory adherence, and enhanced cooperation between researchers, operational staff, quality representatives, veterinarians, and regulatory teams. It underscores the necessity of a unified approach to uphold animal health standards and genetic integrity, directly influencing research validity and reproducibility. To start, we examine the pivotal role of personnel in recognizing and advocating for animal welfare, aligning with both ethical imperatives and regulatory requirements. A detailed overview of institutional and national

animal welfare regulations and guidelines highlights the requisite compliance and collective accountability. This discussion extends into the implementation of the 3R's principle (Replace, Reduce, Refine), promoting alternatives to animal research, optimizing experimental designs to minimize animal use, and refining procedures to mitigate distress.

Preconference Workshop - 4

Occupational Health, Safety, and Emergency Response Management

Dr Samarendra Narayanan, Consultant had delivered about the current scenario that we are living in an era where a plethora of infectious agents and work-related hazards are a source of major concern to all those who work in the laboratory and research domains. Scientists, animals, and caretakers are all faced with the challenge of occupational hazards that can impact adversely their health. Knowledge and application of the principles of occupational health and safety can help mitigate risks of injury, possible life-threatening infections and allergies to personnel. Similarly, knowledge of ergonomic principles can also help increase overall productivity. A functional knowledge of risk assessment and hazard identification for laboratory workers is absolutely necessary for them to be able to deal effectively with these occupational challenges. Thus, knowledge about the importance of engineering controls, administrative controls, PPE is imperative to all connected with the laboratory animal facility and there is a need to constantly review the same. It is an understatement to say that emergencies occur in life with little or no warning. The key is to address and mitigate such emergencies so that a potential disaster instead becomes a manageable emergency, with the least damage to precious human and animal lives, costly equipment, and facilities. The cornerstone of Emergency Response Management is therefore to have a well thought out disaster and emergency response in place and prepared staff who are well-trained in the same. Understanding the principles of Occupational Health, Safety and Emergency Response will undoubtedly contribute to a more effective, safer, and healthier working environment for laboratory animal facility staff, animals, and the community at large.

Monitoring of Laboratory Animal Observations, Daily Housekeeping Tasks, and Contamination Control in Animal Rooms

Dr S P Muthukumar, Chief Scientist, CSIR-CFTRI, Mysuru explained that the effective management of laboratory animal facilities is crucial to ensure the wellbeing of research animals and the reliability of experimental outcomes. A comprehensive approach to the monitoring of laboratory animal observations, daily housekeeping tasks, and contamination control in animal rooms is essential. The study emphasizes the integration of systematic observation protocols, rigorous housekeeping routines, and advanced contamination control measures to uphold the highest standards of animal welfare and experimental reproducibility. The laboratory animal observation component involves the establishment of detailed monitoring procedures, encompassing behavioral, physiological, and environmental parameters. Continuous assessment and documentation of animal health and behavior aid in the early detection of any deviations, enabling prompt intervention and preventive measures. Additionally, the implementation of innovative technologies, such as automated monitoring systems and sensor networks, enhances the precision and efficiency of data collection. Daily housekeeping tasks play a pivotal role in maintaining optimal hygiene within animal rooms. Strict adherence to standardized cleaning protocols, waste disposal procedures, and cage-changing schedules minimizes the risk of disease transmission, ensures a comfortable environment for animals, and promotes the overall integrity of research

outcomes. By integrating advanced technologies, standardized protocols, and comprehensive training programs, researchers can enhance the reliability of experimental data, uphold ethical standards in animal research, and contribute to the advancement of scientific knowledge.

Biosecurity in Barrier Animal Facilities and Research Environment

Dr G H Mohan, Scientific Officer - F and Chief Veterinarian, NCBS, Bangalore described that the Barrier Animal Facilities are the basic necessity for production and maintenance of Laboratory animals in Specific Pathogen Free (SPF) health status. However, mere housing animals in barrier animal facilities will not guarantee SPF health status of animal colonies. Strict, constant and continuous biosecurity measures must be implemented to reduce or eliminate the potential of introducing an adventitious agent into the facility, critical control points that pose the highest biosecurity risk have to be monitored. In that sense, the entry of animals, the use of biomaterials (cells, parasites, viral stocks, purified proteins or antibodies), the cleaning, disinfection and sterilization processes, the housing and husbandry (food, water, air and bedding) and the personnel must be carefully controlled. It is important to carry out regular health monitoring of animals for a defined list of pathogens, environmental microbial quality, proper sterilization and disinfection procedures, standard husbandry activities and assessing quality of materials used in the facility. So, these considerations are important in maintaining the SPF facility for laboratory rodents. He has covered the different risk factors for transmission of agents in SPF facility and the biosecurity measures to control these risk factors.

In addition, he has covered the aseptic procedures for surgery and support including post-operative care monitoring Surgery is commonly performed in laboratory animals used in research, teaching and experiments. Several aspects are to be considered before surgery in laboratory animals. Factors such as animal size, metabolic rate and occurrence of hypothermia are crucial to the success of the surgical procedure. Maintenance of asepsis, and intraoperative and post-operative care monitoring is very important to consider for successful surgical outcomes and survivability of animals. The presentation covered the different types of surgical procedures, aseptic techniques (sterilization & disinfection methods), intraoperative precautions and postoperative care and analgesia methods.

Nutritional Models and Housing for Laboratory Animals Used in Biomedical Research

Dr C Kathirvelan, Associate Professor, TANUVAS delivered a lecture about Laboratory Animal Nutrition which is one of the main areas of animal experimentation and designing of suitable diet is of great concern for experimenters and people concerned with animal ethics/ welfare. The nature and delivery of the diet is of utmost importance for well-being, health, growth and reproduction of the animals. A balanced diet with right ingredients is paramount, which needs scientific and ethical justifications. The other important aspect of laboratory animal experimentation is the research diets. The choice of species depends on the purpose of the experiment and the resources available. Use the species for which the information is desired, in order to avoid the problem of cross-species interpretation. Some dietary studies are performed to gain knowledge applicable to many species. Long growth period after weaning and rapid weight gain during that period. In summary, the nutrition and housing of laboratory animals are crucial in ethics, welfare and experimental points of view and amount to the greater extent in the research output and repeatability.

Improving Animal Welfare Through Advanced Equipment and Caging Systems in Laboratory Facilities

Dr. Manjunathachar H V, Scientist - C, ICMR-NITM, Belagavi emphasised that in the realm of scientific research, equipped animal facilities play a crucial role in ensuring the well-being of laboratory animals. Well-equipped facilities with a range of tools and systems support various experiments and to ensure the health and comfort of the animals. This includes cages, ventilation systems, lighting, watering systems, feeding systems, sanitation equipment, and monitoring and surveillance tools. These components are vital for maintaining optimal living conditions and ethically conducting research. The integration of cutting-edge technologies has led to significant improvements in these systems, encompassing energy-efficient HVAC systems for precise environmental control and advanced LED lighting to mimic natural light cycles. Furthermore, different species and research demands necessitate various types of caging systems to promote and ensure natural behaviors, stimulate mental activity in animals, and facilitate social interactions, thus enhancing the animals' well-being and research validity. These include conventional cages, individually ventilated cages (IVCs), isolators, enriched cages, and group housing systems, each designed to serve distinct purposes. Recent advancements in several areas such as modern cage designs that foster enrichment and social housing systems aimed at encouraging natural behaviors and mental stimulation. Additionally, real-time video surveillance, non-invasive techniques, and data analysis help in early detection of distress or illness (behavioral and health monitoring). The training and socialization of caretakers, coupled with transparency regarding the practices of animal facilities, promise to further enhance animal welfare in the pursuit of scientific discovery.

Main Conference Topics

Plenary Talk - Novel Preclinical Aspects of Cell and Gene Therapy Product Development

Dr K S Rao, Subject Matter Expert - Safety Assessment, Adgyl Life Sciences, Bangalore enlightened the audience as a keynote speaker on the emerging area of novel preclinical aspects. Cell and gene therapies (CGTs) comprise a new class of therapeutics built on the idea that there's no one-size-fits-all approach to treating cancer, immune conditions, and other diseases. CGTs include but are not limited to somatic cell-based therapies, pluripotent cell-derived cell-based therapies, gene- or non-gene-modified or gene-edited versions of these cell-based therapies, *in vivo* gene therapies, organ/tissue-engineered products, and relevant combination products. A key consideration for preclinical toxicology studies for cell and gene therapy is the inclusion of a toxicology study design that mimics the proposed clinical trial design as closely as possible. When possible, the investigational CGT product that will be administered to the patient population should be used in the definitive preclinical studies. The animal species selected for assessment of bioactivity and safety should demonstrate a biological response to the investigational CGT product like that expected in humans. Some factors that should be considered when determining the relevant species include: a) comparability of physiology and anatomy to that of humans; b) permissiveness/ susceptibility to infection, and replication of, viral vectors or microbial vectors for gene therapy; c) immune tolerance to a human CT product or human transgene expressed by a GT product; and d) feasibility of using the planned clinical delivery system/ procedure. "Non-standard" test species, such as genetically modified rodents (i.e., transgenics or knockouts) may be acceptable. More often, safety and effectiveness of the investigational CGT product *in vitro* and *in vivo* can be evaluated in one animal species. Due to

unique features of CGT products, animal models of disease/ injury may be preferable to healthy animals to assess the activity and safety of these products. The use of disease/ injury models provides the opportunity for possible identification of activity-risk biomarkers that may be applicable for monitoring in clinical trials. Taking advantage of opportunities to get feedback from regulators, like the FDA INTERACT (INitial Targeted Engagement for Regulatory Advice on CBER) and pre-IND meetings, can be extremely helpful in planning appropriately. He added that the comprehensive product characterisation with an appropriate testing program demands both *in vitro* and *in vivo* by experienced researchers adhering to the 3R principles.

Session I: Advancements of 3R's in Laboratory Animal Experiments

International Trend in Laboratory Animal Welfare

Dr Tsutomu Miki Kurosawa, Past President of IACLAM and JCLAM, Japan pondered that when we need to understand laboratory animal welfare, we should think of general animal welfare first. There are various international documents in animal welfare available. One of international standard of animal welfare is OIE (WOAH) Terrestrial Animal Health Code Section 7, which describes the animal welfare of various species of animals including agricultural animals, pet animals and laboratory animals. Here we are discussing Laboratory Animal Welfare. There are many international documents in laboratory animals, not only WOAH code, but also CIOMS Guiding Principle (2012), OECD Guidelines (TG405) (2012) and ISO 10993-2 2022 (2022) have been published. Because laboratory animals are used for scientific activities and are intervened by scientists for their primary purposes, laboratory animals should be protected as much as possible. The usage of laboratory animals is always complained by not only animal right groups but also public in general. One of the most sophisticated ideas for laboratory animal welfare is 3Rs（Replacement, Reduction and Refinement). Most of international documents emphasize the importance of 3Rs for laboratory animal usage. Replacement of laboratory animals usage to other means such as cultured cells, computer programs and databases and statistics. In particular, the usage of cultured cells is rapidly advanced and some of testing methods without animals are already recognized by international bodies such as OECD and ISO. This trend will be more rapidly advanced in the near future and we need to catch up this trend carefully. Reduction can be achieved by the usage of above-mentioned testing methods. Particularly the computer-assisted science for the expectation of any toxicological events of medical products with the enormous amount of data collected from previous animal experimentation reduces the usage of animals and animal experimentation. WOAH code stated that "Refinement refers to the use of methods that prevent, alleviate or minimise pain, suffering, distress or lasting harm and/or enhance welfare for the animals used. Refinement includes the appropriate selection of relevant species with a lesser degree of structural and functional complexity in their nervous systems and a lesser apparent capacity for experiences that derive from this complexity." Laboratory animal welfare is equal to the advancement of 3R's.

Refinement of Anaesthesia and Analgesia in Acute and Long-term Rabbit Studies

Dr Patricia Hedenqvist, President, IACLAM, Sweden gave a lecture on anesthesia and analgesia. Rabbits are anaesthetized e.g., in studies of bone regeneration, trauma and cardiopulmonary bypass. Laboratory rabbits are often highly stressed due to limited human contact and need to be trained to handling in a structured manner during 1-2 weeks before surgery. Ahead of transportation to surgery, sedative and analgesic drugs and local anaesthetic cream for later placement of ear

artery and vein catheters can be administered in the housing area. Following the placement of blood vessel catheters and pulse oximetry probe, induction is accomplished by intravenous administration, followed by placement of a larynx mask or endotracheal tube and delivery of oxygen. Anaesthesia is maintained by inhalant and/or intravenous drugs depending on procedure. Monitoring of body temperature, respiration, ventilation and cardiovascular parameters is vital. Postoperative analgesia is planned according to expected pain and administration adjusted to regular assessment. Examples of procedures and drugs used was discussed and differences in approaches between anaesthesia of research. She explained about the positive reinforcement and approach test in rabbits apart from the refinement of multimodal anaesthesia and analgesia monitoring of various routes followed in rabbits.

Using Technology to Monitor Rodent Well-Being

Dr Angela King-Herbert, Head - DTT Comparative Medicine Group, NIEHS, USA elucidated in the presentation the old and new technologies currently being used to monitor the well-being of rodents while in their home cages. Digital biomarkers, home cage monitoring, machine learning, and monitoring physiological parameters of rodents while on study was discussed. The 3R's of digital biomarkers consist of refine animal welfare, reduction of animals and the knowledge gained in study designs utilising translational digital biomarkers was deliberated. She also highlighted about the importance of the team comprises of Information Technology, Cybersecurity, vivarium leadership, data scientist and biostatisticians including the technology provider and publications. Though all of these technologies share some commonalities, the pitfalls, and advantages (positive and negatives) of each monitoring systems were outlined. Two NIEHS studies using some of these technologies were highlighted i.e. Machine vision-enabled behavioural tracking for cross-species extrapolation in Drosophila and mouse models. The parameters were monitored through video and data collected from the subjects was analysed. Similarly, a study was evaluated for the use of physiological monitoring using radio telemetry device during the inhalation studies in rats and data analysis as part of the translational research by following the 3R's principles.

Session II: The 3Rs in Pharmaceutical, Biomedical, Contract Research and Digital Biomarkers

Application of Technology for Advancing Laboratory Animal Sciences

Dr Mary Ann Vasbinder, Site Head, of Integrated Biological Platform Sciences Organization, GSK has emphasised about the recent advances of technologies used for laboratory animals. The application of emerging technologies is providing opportunities to expand the understanding of science and improve the quality of the outcome. The presentation was provided insight into four areas of technology applied to the pharmaceutical industry. Magnetic Resonance Imaging (MRI) is being used across all phases of pharmaceutical development, to include understanding drug distribution, target engagement, pharmacology, and efficacy of medicines. Radio-frequency identification (RFID) is being used as a form of permanent identification of rodents on study. This identification is applied to all future samples and data associated with the individual through barcoding system. The chips significantly reduce the need for animal identification that is not permanent and allows for differentiation between mice within a cage. Two Home Cage Monitoring (HCM) systems are being developed, the Digitally Ventilated Cage (DVC®) and an Artificial Intelligence/Machine Learning powered computer vision system (CV), both

these technologies use AL/ML technology to collect spontaneous activity biomarkers from the animal in the cage relevant to clinical assessments and study related biomarkers; the DVC® can collect information relating to sleep, cage and environmental conditions across hundreds of cages to aid our understanding of how housing and caging conditions impact the animal and for optimizing husbandry practices, while the CV enables a more targeted categorization of mouse behaviors and how they may correspond to the activity biomarkers. Both systems were enabled to have a more powerful toolkit for reproducible and translatable data in the drug discovery process. She has conversed about the animal welfare, operational, scientific, and strategic aspects involved in various capabilities of technology platform.

Full Cost Accounting Not Only Improves Costs, But Also Animal Welfare and Job Satisfaction

Dr Jussi Helppi, President, ICLAS and Head of Biomedical Services, Speaker of Services and Facilities at Max Planck Institute of Molecular Cell Biology and Genetics, Germany talked about the full cost accounting process. Animal facilities face the ever-increasing challenge of running an effective and well-organised programme. The operation of animal facilities is extraordinarily expensive. Those (academic) facilities that have implemented a recharge system for their clients have (mostly) never properly calculated the actual cost of running the facility. The price is often set politically, frequently with nothing more than a guess to satisfy the needs of users and management alike. The actual costs are rarely transparently presented or discussed. This too often leads to misunderstandings about what proper full cost accounting can provide - namely, a perfect tool for managing the facility and motivating animal care staff. We have developed an institute-wide full cost analysis and recharging system to transparently and effectively manage each of our scientific facilities, including the animal facility. By disclosing actual costs, we have understood that the more animal care staff provide experimental services, the more cost-efficient the facility becomes. This benefits our scientists (better and more comprehensive service), our institute (cost-efficient animal facility) and most importantly the animal care staffs (more interesting work, better motivation, integral part of scientific projects). In this presentation, he has explained the advantages of full-cost accounting and gave examples of how to calculate prices. He also showed how this can be introduced as part of global institutional budgeting. Further, explained how transparent full costing leads to better animal welfare, less animal use better-motivated animal care staff, and a facility that is cheaper to run overall. He reiterated that the full cost accounting helped better operations and improved animal welfare by following 3R's principles within the institute.

The Impact of the Animals' Lived Experience

Dr Jennifer Lofgren, Global Head of Animal Welfare and Compliance, Novartis Institutes for BioMedical Research, USA shared her insights on variables and refinements in achieving science. While details such as how much nesting material we place in a cage, how we pick up a mouse during cage change, or which analgesic we administer may be fleeting and minor to *in-vivo* staff, these decisions can result in significant and impactful changes in the lived experience of our laboratory rodents. These impacts are not only to the animals' welfare but also to the research results they produce. While these refinements were once considered nice to have, but far from necessary, published studies have now established that reducing stress associated with suboptimal housing, handling, and post-procedural pain management are important steps to improving laboratory rodent quality of life and the quality and success of the biomedical research to which they contribute. In this presentation, she

has discussed about the experience and literature evidence about the relative impact on a variety of research models of both providing and withholding appropriate enrichment, reducing handling stress, and effective analgesics. Collectively, the balancing variables and refined methods demonstrated the improvements in animal welfare.

Session III - Role of Nonclinical Studies and Process Involved in Biomedical Research

Nonclinical Toxicity Study - How to File an IND application

Dr. Bikash Medhi from PGIMER, Chandigarh elucidated nonclinical toxicity studies to IND application. Nonclinical toxicology is a crucial step in the development of new drugs. A new therapeutic candidate will be introduced to patients in Phase I clinical studies after an investigational new drug (IND) filing aided by toxicity testing. Preclinical Safety & Toxicology, Chemistry & Manufacturing, and other data are included in the IND application to support the start of a clinical trial. Animal Pharmacology and Toxicology studies are usually conducted to assess the safety of the new drug/investigational product for human testing. The duration and nature of the proposed clinical investigations will determine the type, duration, and scope of the animal study that must be included in the application. When planning a toxicological study, considerations for species selection, administration route, and dose regimen are essential. The selection of species is based on the distribution and expression of target receptors relevant to pharmacology. When selecting a species, consideration is given to the patient population, the drug formulation, and the human-specific administration techniques. Identifying and describing target organ toxicity about dose and exposure are the goals of toxicology investigations. Clinicians can use this information to set up appropriate monitoring and to determine whether an adverse reaction would be reversible during a dose-free interval. As a result, recovery groups are frequently used in nonclinical toxicological tests, albeit the length of the recovery period can vary for several reasons. The IND sponsor should also include a statement outlining the locations of the non-clinical investigations and all records that are open for inspection. The nonclinical toxicological investigations in the study must adhere to good laboratory practices (OECD-GLPs), and these procedures must be verified to ensure the validity, integrity, and reliability of data submitted for regulatory inspection and approval, for a successful IND application. The comprehensive talk provided a broader overview right from animal experiments to the successful IND application.

From Genes to Mechanisms: Employing a Genomewide CRISPR Approach in Nonclinical Studies

Dr. Lakshmanan Manikandan, Principal Scientist II, BioPharma Innovations and Solutions, Institute of Molecular and Cell Biology (IMCB), A*STAR, Deputy Program Director, IMCB-Indivumed Joint Lab, A*STAR, Singapore delivered a talk on CRISPR technology. Conventional toxicological studies have struggled to fully elucidate the mechanisms of action of various toxins, and the tissue-specific pathways involved. The advent of the CRISPR-Cas9 system has been a game changer in the field, vastly enhancing our capacity to dissect these mechanisms. This presentation will provide an overview of the genome-wide CRISPR-Cas9 knockout screen that we regularly deploy in our lab for toxicological studies. We will illustrate its application through specific cases where we have utilized this technology to uncover mechanisms of action for known toxins and drugs with toxic side effects. Paraquat, a widely used herbicide, and Nitrofurantoin, a clinically approved agent in common use are known to produce lung toxicity in humans; however, their mechanisms of action at the molecular level are poorly understood. We employed a

genome-wide CRISPR-Cas9 knockout screen using the Brunello library to investigate both paraquat and nitrofurantoin-related lung toxicity. The presentation will delve into the methodologies and findings, showcasing the utility of the CRISPR-Cas9 knockout screen in the field of toxicology.

Session IV - Alternatives to Animal Experimentation: An Overview

Multi Organs-on-a-chip for Disease Modelling

Dr. P.V. Mohanan, Toxicology Division, Biomedical Technology Wing, SCTIMST, Thiruvananthapuram deliberated on alternative methods, especially multi organ-on-a-chip. Microfluidics devices have impactful applications in basic and translational biomedical research due to the peculiar behaviour of fluids in microenvironments. Microchannels and chambers are etched onto biocompatible materials like PDMS where physiologically relevant, organ-specific microenvironments are fabricated. These devices are biomimetic systems built on a microfluidic chip, in which cross-organ communication is established, allowing the study of organ/multi-organ processes and modelling of systemic diseases. The device provides a robust platform for culturing different cells separately under continuous perfusion. The complexity of these devices can vary from a single-cell device (Organ-on-a-chip) to Multi organs-on-a-chip (MoC). The MoC technologies mimic organ interactions observed in the human body and lead to the absorption, metabolism, excretion and toxicity of the molecules of interest. Several technologies are employed to fabricate microchannels and chambers that allow fluid flow even in ranges of femtoliters in a tuneable manner. The fast-track advancements in microfluidics has initiated novel concepts and developments in many fields of science. Micro-engineered devices, incorporating fluidics and sensor technologies, have permitted the transfer of 3-D tissues to a dynamic environment, creating organs-on-a-chip that more closely resemble human organs in vivo. 3D bioengineered constructs, ex vivo tissues, re-cellularized scaffolds, and bio-printed constructs with micro-fabricated structures, and possibly chemical, physical, or molecular sensors for real-time on-chip assays are integrated inside these microchannels to achieve multi-organ-on-a-chip platforms. Major Applications of MoC are toxicity screening, drug metabolism, pharmacokinetics, ADME profiling, cancer studies or other disease modelling.

Development of Human iPSC-derived 2D and 3D models for Toxicity/ Safety Assessment: The Indian Perspective

Prof. AB Pant, Senior Principal Scientist & Group Head, System Toxicology & Health Risk Assessment Group CSIR-IITR, Lucknow elaborated on 2D and 3D models in safety assessment. Our understanding of organ function and development has only grown in the last century due to using a large diversity of non-primate model systems ranging from Drosophila to Zebrafish and rodents. While these models successfully demystified the general principle of human development but failed to translate in humans. Two-dimensional (2D) cell cultures have revolutionized research, although transformed cell lines have genetic expression and metabolic contours not indicative of human bodily systems. 2D cell cultures obtained from induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs), on the other hand, have transcriptional profiles that are very similar to those of human cells, yet cells in 2D flatline to mimic the three-dimensional (3D) properties of tissue architecture. For decades, human organ-specific model formation under *in vitro* conditions was an insurmountable challenge. The discovery of iPSCs and their re-programming and self-organizing properties have facilitated the creation of various

organoids/ spheroids. Further, the technological advancements in cultivation, bulk production, differentiation, and cytosolic and genetic transformation of immortalized and primary cells derived from human and animal origin in 3D cultures have provided an edge in developing high-throughput screening models for testing drugs and chemicals. The potential applicability of human iPSCs is also being looked at as restorative medicine by replacing the specified damaged tissues with stem iPSC-derived 3D systems. Researchers in India have had tremendous success restoring various degenerative disorders with special reference to neuronal disorders, spinal cord injuries, aplastic anemia, acute myeloid leukemia, etc., by transplantation of iPSC/ stem cell-derived specific cell types in experimental models and limited clinical trials. Industry and academia joint efforts have also culminated in the less complex, unique 3D characterized spheroid epidermis model using two major cell types present in the epidemic, i.e., keratinocytes and melanocytes. This human epidermis mimicking model was found to be suitable for studying the complete melanogenesis pathway and transfer of melanin from melanocytes to physiologically differentiated keratinocytes within the 3D spheroids microenvironment. Nevertheless, a long list of questions is still to be answered. The pace of iPSC/ stem cell research indicates that a rationalized remedy to cure each individual's disease will be possible someday using specified desired cells derived from human iPSCs. These alternative methods could minimise the usage of animals to some extent depending upon the context and type of research.

Understanding the Biomechanism of Regeneration and Degeneration in Alternate-model Animals

Dr. M Mohammed Idris, Senior Principal Scientist, CCMB, Hyderabad delivered a talk on exploring the biomechanism of regeneration and degeneration in alternative animal models which holds promise for enhancing our understanding of these underlying processes. The research group focuses on unraveling these mechanisms in non-traditional model organisms, such as zebrafish, geckos, echinoderms, tunicates, and acoel worms. Our approach utilizes high-throughput genomics and proteomics techniques to shed light on the role of various genes and proteins in regenerative environments. This investigation is particularly significant as it could pave the way for transforming non-regenerating systems into regenerating ones, offering potential therapeutic applications. One fascinating example of our research involves the epimorphic regeneration of zebrafish caudal fin tissue, a complex and complete process. Through comprehensive analyses, including high-throughput transcriptomics and quantitative proteomics using iTRAQ, we identified 1408 differentially regulated genes and 661 proteins during zebrafish caudal fin tissue regeneration. Notably, our findings revealed the involvement of PRMT, SLC, Interleukin, HOX, Neurotransmitter, and several novel genes in the regeneration process, as evidenced by their differential regulation. Furthermore, our network and pathway analyses unveiled connections between the differentially regulated genes and proteins and key biological processes. These processes encompassed cell cycle control of chromosomal replication, nervous system development, and cellular development, growth, and proliferation pathways. Our study provides a comprehensive and detailed insight into the gene and protein changes occurring during tissue regeneration, enhancing our understanding of this intricate phenomenon.

Session V - Modern Husbandry, Refinement Methods, Enrichment Program and Management

Human-Animal Interaction and its Implications for Animal Welfare and Research

Dr Samarendra Narayanan has elucidated that the interactions between humans and animals have existed since pre-historic times. The history of human-animal relationships has been broadly categorized into three stages, a pre-domestic era, in which human societies were hunter-gatherers who did not perceive a difference between themselves and other animals; a domestic era characterized by the development of beliefs in the difference and superiority of humans; and a post-domestic era, where many in the population have little direct experience of animals. People have an emotional need to connect with animals, which is explained by the Biophilia hypothesis which focuses on our innate tendency to focus on life and life-like processes might be a biologically based need. The scientific study of human-animal interaction (HAI) and the human-animal bond is known as anthrozoology. HAI occurs in diverse settings such as in zoos, the laboratory animal environment, the home, agriculture and the wild. Human Animal Interactions such as non-aversive handling methods, positive human attitudes and aptitude towards animals can reduce background stress and anxiety in laboratory animals, provide animals with a congenial environment, and reduce the chances of these variables confounding experimental results. In our endeavour to balance the quest for scientific knowledge, with the welfare and rights of animals, we must be mindful of the "Three Rs" principle at all times in order to minimize harm and suffering to them. HAI in research can thus profoundly influence the quality and reliability of scientific experiments. Its ramifications extend into the future, where technological advancements, shifting societal attitudes, and innovations in alternatives to animal testing underscore a growing commitment to shaping a future where animals are treated with compassion and respect, and where scientific progress coexists harmoniously with ethical responsibility.

Implementation of 3R Principles in Pharmacokinetic Studies

Dr. Amol Raje, Lead Scientist, Syngene International Ltd, Bangalore delivered a talk on pharmacokinetics. Mice are commonly used for Pharmacokinetic (PK) studies during early drug discovery projects. Due to physiological limitations of blood withdrawal from mice, sparse/discrete blood sampling is widely used leading to increased usage of the mice. With the advancement of analytical technologies, sample volume requirement has drastically reduced. Micro-sampling (collection of 20-30 µL of blood) is an efficient technique of blood collection for PK study. Micro-sampling significantly reduces the usage of animals in PK studies. Dry blood spot (DBS), one of the micro-sampling techniques is gaining more popularity as it offers many advantages over other conventional analytical techniques. Micro-sampling can be effectively applied to rats as well. The sampling from saphenous veins in rats reduces the surgery stress to the animals. During early discovery stage, many new chemical entities (NCE) are screened in the pharmacokinetics study. Cassette dosing at early discovery stage helps in the reduction in animal usage without compromising the quality of data. One of the components of the PK study is estimation of concentration in target tissue. For determining tissue concentrations, generally, animals need to sacrifice for tissue collection. Microdialysis technique can be used for tissue distribution study where terminal sacrifice of animals is avoided. Additionally, with the use of microdialysis technique, estimation of unbound/free concentrations of NCEs at target site along with biomarker concentrations can be analysed. Use of fluorescent imaging or Quantitative Whole-Body Autoradiography (QWBAR) are an alternate option for evaluating tissue concentrations. Use of advanced technologies in Pharmacokinetics studies helped in reducing and refining the conventional procedures of Pharmacokinetics studies which are in line with the 3R principles.

Session VI - Improving Research Reporting on Scientific Communications and Laboratory Animal Experimentation

ARRIVE and RIVER: NC3Rs' Guidelines to Promote Responsible Reporting

Dr Nicola Foster from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK, outlined on how the ARRIVE guidelines can be used to ensure rigorous reporting of *in vivo* studies. She also briefed about the RIVER, a new set of reporting standards for *in vitro* studies to be introduced. She has stated that incomplete reporting is a major contributing factor to the poor reliability of many preclinical studies. The talk provided insights on the 3R's and applications of the ARRIVE guidelines by improving the reporting of scientific results.

Progressing on the 3Rs Journey

Dr Jessica Eddy of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK, pointed out that covering the NC3R's resources which help researchers, animal care staff, and vets, as well as other members of the scientific community, put the 3Rs into practice. This topic detailed the evaluation of environmental enrichment, non-aversive mouse handling, and the 3R's self-assessment tools.

Limitations of Non-Human Primates Usage in Biologics and Biosimilars Drug Development for Predicting Efficacy and Toxicity in Clinical Trails

Dr. KVS Narayana Raju, Head, Pre-Clinical Pharmacology & Toxicology, Biologics, Dr. Reddy's Laboratories Ltd., Hyderabad deliberated on biologics and animal models. The use of nonhuman primates (NHPs) in nonclinical toxicity testing is based on the regulatory requirement that the toxicity of new medicines should be tested in animals for safety and tolerability prior to first-in-human (FIH) trials and to support long-term dosing in humans. The non-clinical safety evaluation of medicinal products intended for human use is generally tested in two mammalian species, a rodent and a non-rodent for large-molecule biopharmaceuticals. The purpose of these studies is to identify potential target organs for toxicity and to establish a dose at which no adverse effects are observed. Although toxicity studies for biopharmaceuticals are frequently conducted in NHPs as the only pharmacologically relevant species, pharmacology models of disease are infrequently conducted in NHPs because of the ethical considerations of developing these models in NHPs, the expense of the studies, the high inter-animal variability and the low power of the studies to detect clinically meaningful changes. Therefore, pharmacology studies that support the use of a monoclonal antibody (mAb) in human disease are often conducted using rodent mAbs (surrogate mAbs) that bind to the analogous target protein in rodents. General considerations for NHPs should be used only when they have been shown to be relevant based on data, and these studies often provide the fundamental supporting information that directs the indication, dosing, and regimen in human trials. With careful planning, conducting fewer overall studies and using fewer NHPs may be many times preferred, by worldwide health authorities. Opportunities exist within ICHS6(R1) to reduce the number of studies performed for biotherapeutics, for example by incorporating of cardiovascular endpoints into other toxicology studies. If the NHP is the only relevant species, then developmental toxicity assessment in this single species is sufficient rather than studies in two species; In vitro technologies can be used to understand the potential for immunotoxicity, as well as other safety concerns, as part of both candidate selection and the safety assessment process Biosimilars are the products that are highly similar to biologic reference products and there are no clinically meaningful differences

regarding quality, biological activity, safety and efficacy. The approval pathway of biosimilars includes a stepwise approach starting from nonclinical analyses such as *in vitro* physicochemical, structural, functional and toxicological similarities and if there are still some minor differences between the products in some of the non-clinical attributes, outstanding discrepancies should be addressed by the clinical trials. There are currently major differences between global biosimilar regulations, with ambiguity in wording leading to variability and conservatism in the nonclinical toxicity testing performed. The EU guidelines outline a tiered approach starting with *in vitro* binding/biological activity studies before 'determining the need for *in vivo* studies. It is therefore possible to develop a biosimilar with *in vitro* data alone, but if an *in vivo* study is necessary 'the conduct of repeated dose toxicity studies in non-human primates is usually not recommended. However, the conduct of nonclinical *in vivo* studies is often driven by anticipated or real requests from regulatory agencies or institutional ethical committees. Other reasons for conducting nonclinical *in vivo* studies include an inability to meet with regulators promptly, inconsistent approaches between geographic regions or within the same geographic region, default practice to carry out nonclinical *in vivo* studies within the sponsoring company to provide a comfort factor for the sponsoring company to meet the global regulatory requirement with a global toxicity study design.

Session VII - Regulatory Framework and Animal Experiments in Global Scenario

AAALAC International Accreditation Program Updates

Dr Montip Gettayacamin, Senior Director for Southeast Asia, AAALAC International, Thailand updated that the AAALAC International is a private, non-profit organization that promotes the humane and ethical treatment of animals in science through voluntary program assessment, accreditation, and education. AAALAC International has been recognized around the world as a symbol of high-quality animal care and use for research, teaching and testing, as well as promoting animal welfare and maintaining safety. About 1,100 institutions in 50 countries have earned AAALAC accreditation. There are more than 250 accredited programs in Asia and Australia. The AAALAC International's Council on Accreditation evaluates overall performance and all aspects of any animal care and use program, involving an in-depth, multi-layered, confidential peer-review process. The AAALAC evaluators (site visitors) consider compliance with applicable local animal legislation of the particular country, institutional policies, and use a customized approach for evaluating overall program performance using principles outlined in the Three Primary Standards: Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020), or European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes, Council of Europe (ETS 123), and supplemental Reference Resources as applicable. This presentation outlined the AAALAC International accreditation program highlights, the strategic plan and administrative updates.

India's Journey to OECD MAD and Towards Excellence

Dr. Ekta Kapoor, National GLP Compliance Monitoring Authority, Department of Science and Technology, New Delhi provided an overview of OECD with special reference to Mutual Acceptance of Data (MAD). Many countries globally mandate the conduct of non-clinical and environmental fate studies meant for regulatory submissions in compliance with the quality system of Good Laboratory Practice (GLP). The genesis of the GLP in the 1970s and the recommendation of the Organization for

Economic Cooperation & Development (OECD) Council for the adoption of the Principles of GLP by member countries in 1981 for Mutual Acceptance of Data (MAD) were of great significance in safety evaluation of chemicals and other test items. Since then many OECD member countries started adopting the OECD Council decisions. Considering the importance of the GLP quality system for data generation to meet global requirements, certain Indian industries & CROs established GLP test facilities in the early 1990s and received GLP certification from the monitoring authorities of a few OECD member countries namely Germany, The Netherlands, and Belgium. The initiatives of the Indian industry in setting up GLP-compliant test facilities as well as the OECD Council decision of 1997 concerning the adherence of non-member countries to MAD led the Indian government to establish the National GLP Compliance Monitoring Authority (NGCMA) in 2002 under the Department of Science and Technology. The genesis of the NGCMA and its subsequent role in compliance monitoring, including certification of test facilities in India, provided opportunities for the then-existing and new test facilities to conduct studies for global and Indian sponsors. Continuous efforts of the NGCMA resulted in India achieving the coveted global recognition of Full Adherence status for MAD by the OECD Council in 2011. Her presentation covered an overview of the genesis of the NGCMA, key roles such as compliance monitoring in India and representing the country in the OECD Working Group, inspection and certification processes, achievements over the past one and a half decades and growth of GLP-certified test facilities in the country. The talk has provided regulatory information and OECD processes.

CCSEA - Aims, Rules, Functions, and Recent Notifications of CCSEA for Nominees and Organizations

Dr Vivek Tyagi, Expert Consultant, CCSEA, New Delhi deliberated that the experimentation on animals in India is regulated by the Committee for Control and Supervision of Experiments on Animals (CCSEA), a statutory body constituted under Section 15(1) of the Prevention of Cruelty to Animals Act, 1960. The subject comes under the Ministry of Fisheries, Animal Husbandry & Dairying, Government of India. The establishments engaged in experiments, breeding, and trading of laboratory animals are required to act in conformity with the provisions of the Prevention of Cruelty to Animals Act, 1960, and "Breeding of and Experiments on Animals (Control and Supervision), Rules, 1998 which were further amended in 2001 and 2006. The functions of CCSEA are as below: i. Registration of establishments engaged in Breeding of animals and conducting experiments on animals and Renewal of registered establishments. ii. Constitution, Re-constitution and Revision of the Institutional Animal Ethics Committee. iii. Approval of Animal House Facilities for Small and Large animals. iv. Permission for conducting experiments on large animals. v. Conducting conference, seminars, workshops, nominee trainings etc for the awareness of laboratory animal welfare.

Session VIII - Laboratory Animal Research and New Approach Methodologies (NAMs)

Animal Models for a Mechanistic Understanding of Disposition of NCEs

Dr. T. Thanga Mariappan, Scientific Director, Bristol Myers Squibb R&D Center, Bangalore delivered a talk on animal models concerning disposition. Preclinical investigation in animals provides invaluable information for discovering new chemical entities (NCEs). In the discovery of NCEs, the disposition of the compounds in the *in vivo* system leading to required systemic exposure is one of the important considerations in developing successful drugs. Several NCEs elicit poor exposure due to various hurdles during the disposition. It is important to understand the reason(s) for the poor exposure and use the information to optimize disposition

of the compounds during early discovery. This presentation will explain a few important rodent models that help mechanistically understand the disposition of NCEs.

Current Status of Acceptance of NAMs by Regulatory Agencies

Dr. Varun Ahuja, Head-Toxicology, Safety Assessment, Bangalore has provided updates on regulatory perspectives concerning NAM. Regulatory toxicology applies the knowledge of toxicology to evaluate the safety of chemicals and help to ensure that society benefits from their use without unacceptable risks to human and animal health and the environment. Data from traditional animal toxicity test methods have been used for many years to inform human health hazard identification and risk assessment. However, studies relying on animals to characterize the effects of chemicals can be of questionable or limited biological relevance to human effects. New approach methodologies (NAMs) are increasingly being used for regulatory decisions because of their potential to reliably and efficiently produce information that is fit for purpose while reducing animal use. NAMs are defined as any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment without the use of animals, including in silico, in chemico, *in vitro*, and *ex vivo* approaches. Regulatory agencies have already accepted various *in vitro* NAMs such as 3T3 NRU phototoxicity test (OECD 432), skin sensitisation test methods such as ARE-Nrf2 Luciferase test (OECD 442D) and h-CLAT (OECD 442E) etc. In silico NAMs are also accepted by regulatory agencies including for assessment of mutagenicity of pharmaceutical impurities (ICH M7-R1, 2017). A combination of in silico and *in vitro* NAMs called as a 'Integrated Testing Strategy' has been accepted by regulatory agencies for the assessment of sensitisation potential of chemicals (OECD 497). The REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation in Europe specifically promotes the use of in silico prediction (e.g. QSAR and read-across methods) as an alternative to animal testing. ECHA's 4th Report on the Use of Alternatives to Testing on Animals for the REACH Regulation confirms that results from alternative methods continue to be used over and above new animal tests in dossiers submitted for REACH (ECHA, 2020). The path forward looks challenging but quite optimistic.

Mimicking Human Disease in Animal Models

Dr. Shailesh Dudhgoankar, Scientific Director, Bristol Myers Squibb, India Ltd, Bangalore deliberated that the animal models of human disease provided invaluable information for defining disease pathophysiology, genetic mechanisms, and novel therapeutic approaches. Historically, the use of animals in biomedical research and drug discovery is based on the notion that basic processes, both anatomical and physiological are sufficiently similar across the species which allow us to extrapolate findings from one species to another. Animal models are important tools to evaluate novel therapeutics. However, the translatability of human disease has been questioned multiple times. Ideally, to a larger extent animal models should replicate human disease phenotypes. However, most of these models display many but not all disease phenotypes. Similarly, these models cover part of the underlying mechanistic aspects but does not mimic entire spectrum of mechanistic biology. These factors pose challenges for the selection of the right / appropriate animal model to study disease biology or to evaluate the pharmacological effect of novel chemical entities. These challenges can be managed by using multiple models for a disease indication. Furthermore, the humanization of the mouse model is another important aspect being considered to overcome the challenges related to species-specific differences. Humanization of mice allows us to evaluate the impact of test

compounds or test antibodies on human target (protein of interest) in a mouse setting.

Session IX - Technical Trade Presentations on Animal Models and Technology Platforms

Empower Your Research with the VevoF2 LAZR-X Photoacoustic Imaging Platform

Dr. Shripad Bangale, Regional Application Specialist, Fujifilm Visualsonics Inc, has explained that FUJIFILM VisualSonics specifically focuses on developing ultrasound technology that has been scaled to much higher frequencies than commonly found in many of the conventional ultrasound systems on the market today. As a result, our ultrasound platform provides images at resolutions that far exceed any other system available on the market; as fine as 30 micrometers, clearly differentiating our company from our competitors. We originally introduced our technology in the area of preclinical research as micro-ultrasound, specifically in small animal models of human disease (e.g. mice or rat models). This coincided with the explosion of the human genome project (circa 2000), where numerous genetic models of human disease were developed in small animals. The study of these disease models benefited from the use of imaging techniques to follow the disease progression in vivo, as opposed to using ex vivo methods such as histology. By using ultra-high frequency, researchers were able to use our technology to study their live animals in real-time, longitudinally, and with no issues of safety or side effects. Our preclinical customers are mostly academic researchers, often involved in the fields of cardiovascular and cancer research. Neurobiology and developmental biology are other key areas that are among an ever-growing number of applications. For these customers, funding for purchasing capital equipment comes mainly through grants. Obtaining funding is often a challenge and sources of funding may change from time to time, so we constantly need to be aware of these dynamics and help potential customers navigate through this. The imaging modalities helps understand the system in a real-time manner by the advancement of technologies.

Observing the Invisible Anatomy: Molecular Imaging by Multimodality & Their Advancements in Small Animal Research.

Dr. Shahzada Asad, Principal Product Specialist, INV segment-Revvity Life Sciences, delivered a lecture on molecular imaging. Advances in non-invasive *in vivo* imaging techniques have raised the use of animal models in preclinical drug discovery and development to a new level, enabling quick and efficient drug efficacy screening. Live animal imaging delivers fast, longitudinal, accurate, real-time and quantitative assessments of molecule efficacy. PerkinElmer IVIS® In vivo Imaging Systems are versatile and advanced in vivo imaging system available on the market today. From one Planar 2D to 3D imaging offers true 3D tomography for both fluorescent and bioluminescent reporters that can be analysed in anatomical context against a Digital Mouse Atlas. On the other hand, µCT which provides quantitative high-resolution 3D and 4D volumetric data non-destructively, the applications of morphological and functional imaging can be integrated into the routine practice of a small imaging laboratory or imaging core facility. With a focus on the practical aspects of µCT imaging cardiovascular, pulmonary, kidney, bone, metabolic, and oncologic disease how image acquisition, & interpretation but complementary to theses imaging technologies hands-free, automated, high-throughput preclinical ultrasound imaging system that delivers high-resolution 2D and 3D ultrasound images in just a few minutes. This innovative in vivo ultrasound system removes the challenges associated with conventional hand-held systems & edges on multiple applications like oncology, tissue stiffness, cardiac, renal disorders, etc.

Creating a Stimulating and Cooperative Environment for Göttingen Minipigs

Dr. Susi Soegaard from Ellegaard Göttingen Minipigs A/S spoke about minipigs as animal models. When developing behavioral management and husbandry procedures for laboratory minipigs, it is important to recognize that minipigs are highly intelligent animals with a unique perspective on their environment. This intelligence might cause a challenge to the provision of a stimulating and appropriate environment. This presentation will give examples on how to stimulate natural behaviors such as rooting, farrowing-related nest building, play and exercise by providing species-specific and appropriate environmental enrichment. Natural products such as straw and hay prompt and stimulate natural behaviors and can be used in a variety of ways to maintain novelty and remaining interest. Various toys and homemade devices such as ice cubes with or without flavor are a good supplement to engage minipigs in short-term active behaviors. Daily socialization and positive human interaction are furthermore an important part of creating a stimulating and cooperative environment for minipigs. The recommendations presented are based on years of in-house observations and experience from breeding, housing, and socializing several thousand Göttingen Minipigs in a barrier breeding environment. The socialisation helps to improve the animal welfare.

Session X - Laboratory Animal Health Monitoring and Considerations for Implementing the Quality Control Program

The Practical Point to Consider in Use and Care of SPF Laboratory Animal

Dr. Richard C Lee shared his experience on maintaining the SPF animals. The talk covered the practical points of consideration such as WHY do we need SPF animals; What factors are involved in the better care and use of SPF animals and HOW to plan and implement properly in the laboratory animal facility. The lecture has provided insights on colony management and successful breeding program with high-quality animal care and welfare.

Caring of Immunodeficient Mice: From Nude to NSG

Dr. Charu Gupta, Technical Information Scientist, The Jackson Laboratories, USA provided inputs on caring for immunodeficient mouse models. Mouse strains with varying degrees of immunodeficiency are powerful tools for modelling human disease. She has discussed about the most widely used immunodeficient mouse models, from Nude to NSG mice, and essential considerations for selecting the most appropriate model. The selection criteria include and not limited to, 1. Identifying which model could be best for your research application based on immune depletion phenotypes, disease and cell response; 2. Identifying the types and signs of common infections that NSG mice are prone to and common reasons contributing to mortality. Discussing opportunistic, pathogenic, and commensal microorganisms; 3. Understanding how to care for immune deficient NSG mice-recommended housing conditions and husbandry processes for successful care of these immunocompromised mice. The talk has offered a better understanding of caring for the immunodeficient model at large colonies

Conclusion of Conference Proceeding

The 11th International Conference (LASACON-2023) of LASA India has provided a platform where at least 40 invited eminent professionals have delivered scientific talks, and provided technical information apart from oral presentations (18) by the participants including poster presentations (121) displayed by the students and researches and facility managers. The three-day event (pre-conference workshop and main conference) was successfully executed by the organising committee and the

valedictory function was graced by Dr Chetan Tamhankar, Vice President, Syngene, duly acknowledged the collaborators, speakers and awarded the winners for oral and poster presentations. Collectively, the participants had an opportunity to interact with the eminent speakers, technology partners and delegates towards promoting humane care and use of laboratory animals, thus enhancing the scientific outcomes of results in research and testing.

Conflicts of interest The authors declare No Conflicts of Interest

Author Contributions The authors contributed at various phases of the event such as scientific committee, planning and execution, conduct of sessions and networking with speakers and delegates.

Funding Laboratory Animal Scientists' Association (LASA), India.

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