Meeting Report

First National Congress on Alternatives to Animal Testing and Post-Congress Workshops in India

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The Doerenkamp-Zbinden Foundation-sponsored Mahatma Gandhi-Doerenkamp Center (MGDC) for Alternatives, India, together with Episkin Academy, France, conducted four editions of the "*Handling of Reconstructed Human Epidermis Model*" training program in India during 2012-15. An alliance has now been formed between (i) Episkin Academy, (ii) Government of India Department of Science and Technology-supported "National Facility for Biopharmaceuticals" located at G. N. Khalsa College, Matunga, Mumbai, and (iii) EnvisBE Solutions Pvt. Ltd., a Contract Research and IPR Company, Dadar (West), Mumbai, together with the former Doerenkamp chair in India, towards furtherance of service to India in the context of alternatives. The alliance partners launched the *First National Congress on Alternatives to Animal Testing*, indicating that there could be more congresses to come. The objective of the congress, convened on December 14, 2017 at Hotel Orchid, Vile Parle (East), Mumbai, was to provide a platform for sharing information and updates on the latest practices in safety assessment as well as regulatory guidelines that are emerging in the drug discovery, environmental risk assessment, and cosmetic sectors. Dr Kiran Mangaonkar, Principal of G. N. Khalsa College, inaugurated the Congress when Dr Ajith Singh, member of the Management Committee of the College, offered felicitation. More than 100 delegates, who represented academic, research and industrial communities, took part.

Delivering the key-note address on "*Trends in* in vitro *approaches to toxicology and pharmacology*", Prof. Mohammad A. Akbarsha, former Doerenkamp chair in India and founder-MGDC, elaborated on the academic, technical and regulatory perspectives of adopting the alternative modalities of chemical risk assessment as well as drug discovery processes, and developing newer testing strategies. Advances in molecular biology have revolutionized toxicological risk assessment with rapid, less expensive and highly relevant *in vitro* and *in silico* systems. *In vitro* assays form the core of alternative tools, which include 2D and 3D cell cultures and the more superior microfluidic systems that have emerged as the microphysiological systems patronized by EPA and FDA that offer highly appropriate endpoints that closely simulate/reproduce organ-specific functions with sensitive multi-parametric endpoints. The latest imaging technologies, supported by omics-coupled high-throughput systems, improve the non-invasive exposition of molecular and cellular processes and provide high sensitivity to assays, which are quantified using robust algorithms. The various avenues now available for assays/screens include i) monoculture of primary cells or established cell lines in the context of screening chemical entities; ii) different levels of co-culture (including multiple organ co-culture); iii) stem cell applications in *in vitro* pharmacology/toxicology; iv) 3D culture (including organ-on-chip, multi-organ chips, and human-on-chip to decipher cross-talk between cells and between organs); and v) introduction of robotic approaches and high-throughput screening. Sharing his experience at the Asian Congress (2016), World Congress 10 (2017), and American Society for Cellular and Computational Toxicology (2017), he emphasized the need for India to catch up with the developed and the rest of the developing nations in practicing the available alternatives and contributing to the global alternatives movement.

Dr **Christian Pellevoisin**, Scientific Director, EPISKIN Academy, France, spoke on "*Reconstructed skin models and methods for hazard and risk assessment of chemicals and cosmetics*". He explained that progress has been made in toxicology for lower-tier endpoints with successful regulatory-accepted methods such as *in vitro* skin irritation/corrosion testing using reconstructed human epidermis (RHE) and, more recently, *in vitro* eye irritation with reconstructed human corneal epithelium (RHCE). Other methods for genotoxicity or skin sensitization testing are in the process of validation, and many are already used for routine screening to assess permeation or phototoxicity. Combined in Integrated Approach to Testing and Assessment (IATA) with *in silico*, read-across and weight-of-evidence (WoE) approaches, they allow full replacement of animal tests for several endpoints. These achievements concern not only chemical or cosmetic industry but also biocide testing or drug development. The recent success of the round robin study for skin irritation of medical devices with RHE models opens the way for ISO 10993 standards revision. There is still a need to develop non-animal methods for further endpoints and to support them through validation, regulatory acceptance and worldwide implementation.

"*Cosmetic safety testing – The Indian scenario*" was the topic of presentation of **Benedict Mascarenhas**, Chairman & Managing Director, EnvisBE Solutions Pvt. Ltd., also member, Cosmetic Sectional Committee, BIS, India, and Convener

of Work Group on Safety Testing of Cosmetics under PCD 19. Owing to tremendous growth of the cosmetic industry in recent years, both in size and complexity, rapid changes in cosmetic regulations in India and elsewhere have made it imperative for formulators to have a much greater understanding of the regulatory climate within a country in order to build and sustain successful brands. With the EU, Israel, India, Norway, New Zealand, etc. imposing a ban on animal testing for cosmetics, and some other countries actively contemplating the same, cosmetic industry realizes the urgent need to incorporate validated alternatives in their safety assessment program. Skills associated with category-specific product safety assessment of formulations, raw materials, ingredients and additives as well as toxicological risk characterization, exposure and risk assessment are emerging requirements. *In silico* data mining and data gap analysis coupled with validated alternative *in vitro* techniques in IATA will see increased use and also bring to the fore the role of the safety assessor in charge of product safety evaluation.

Prof. **Achuthsankar S. Nair**, Computational Biology and Bio-informatics, University of Kerala, Trivandrum, spoke on "*Bioinformatics – The computational microscope*". Bioinformatics, the branch of life science that attempts to unravel the secrets of cellular life by analyzing the information embedded in DNA, RNA and proteins, is indirectly a great contributor to the field of alternatives to animal experiments, such that even a simple gene-finding tool available on the web is an alternative to a possibly cruel gene knock-out experiment in a wet lab. By providing strong hypothesis to the experimenter, bioinformatics reduces the number of animal trials to a great extent. In his elaboration, he focused on the role of powerful algorithms in serving as computational microscopes to replace animal experiments.

Dr **Shruta Dadarkar**, Senior Toxicologist, L'Oréal Research and Innovation, Shanghai, China, speaking on a "*New paradigm for toxicologist in risk assessment of cosmetic products*", opined that consequent to the animal testing ban for cosmetics in many countries, adoption of modern testing approaches has become crucial. The challenge is to maintain a high level of robust safety evaluation in the absence of animal data. Every toxicologist must adapt and re-orient his/her evaluation strategy in this new paradigm. Not individual but rather the collective use of different methods including *in silico* analysis and *in chemico* and *in vitro* tests is the way to go. Standardization and validation of the newer methods in the pipeline will pave the way to create better decision trees for mitigating risk and justifying the safety of marketable cosmetics.

Dr **Adip Roy**, Head, Regulatory Affairs, Amway India Enterprise, Gurgaon, India, spoke on "*Progressing non-animal approaches to safety assessment*". He emphasized that assuring complete consumer safety of novel ingredients without generation of any animal data is a considerable challenge. Assessing safety using non-animal methodologies implies developing novel toxicological risk assessment strategies involving a fundamental change in the way safety assessments are carried out. There has been significant progress in the development, validation and acceptance of non-animal approaches to toxicological risk assessments as well as developments in exposure science that can help reduce the number of animals used for safety assessment. Toxicology and ecotoxicology, which underpin consumer and environmental risk assessment, are evolving rapidly. Effort is being made in the context of OECD's "Adverse Outcome Pathway" (AOP) program, where work is in progress to put in place the tools and novel thinking needed to implement Toxicity Testing in the 21st Century/AOP-based consumer and environmental risk assessments. Although significant expertise exists in Indian science (e.g., in the areas of bioinformatics, modelling, *in vitro* toxicology, "omics" technologies) to contribute to this novel science, to date there has not been significant coordinated development in research on alternatives to animal testing. Academia, government organizations, industry and regulatory authorities in collaboration should contribute to developing alternatives to animal models for toxicological assessments.

Elisabet Berggren, from the Joint Research Centre of the European Commission, in her recorded talk on "*Integration of new approach methods for testing and assessment*" said that EURL ECVAM is endeavouring to develop, evaluate, harmonize and promote innovative non-animal test methods for the regulatory safety assessment of chemicals. It is now generally accepted that no more animal testing is required for topical toxicity testing, and major resources are employed to support the development of alternative methods in an IATA to tackle systemic toxicity. It is possible to develop 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. People at EURL ECVAM provide support to a broad range of policy areas including industrial and household chemicals, cosmetics, food, plant protection products, endocrine disrupters and chemical mixtures, and work on strengthening knowledge exchange to progress the 3Rs between different areas of expertise and on developing education on how to apply new scientific solutions to protect human health and the environment.

The last session of the Congress was a panel discussion moderated by Benedict Mascarenhas. The panelists included Dr Mohammad A. Akbarsha, Benedict Mascarenhas, Dr Christian Pellevoisin, Dr Adip Roy, Dr Shruta Dadarkar and Dr Dipti Kapoor (Science Policy Advisor, PETA India). The questions covered the implementation of alternative methods in India including the availability of cost-effective options, role of toxicologists and career opportunities in toxicological research as well as opportunities for India to play a significant role in the global validation programs that are underway in the area of alternate methods to safety testing. Following are the highlights of the discussion:

- According to a recent European survey the costs of *in vitro* assays for local endpoints, such as skin irritation/corrosion, are in the same price range as those of *in vivo* assays; *in vitro* assays are often faster, mechanistically closer to the human

situation thanks to the use of human cells and tissues and are quantifiable in contrast to expert-based scoring of animal methods.
- Shifting from *in vivo* to *in vitro* requires a high level of confidence and reproducibility in the experimental models. In tissue engineering this can be achieved only with huge investments for tissue production, quality controls and validation for regulatory acceptance.
- Recent developments in the cultural specificity of India with regard to cows would limit access to the BCOP for eye irritation. This can be conveniently replaced by other validated methods such as ICE or STE.
- Alternative methods provide significant opportunity to those who are interested in making a career in toxicological research.
- Industry is open to participate in and support initiatives in the area of research and validation of non-animal methods.
- There is a pertinent need to launch an Indian Society for Alternatives to Animal Experiments.

Post-congress training workshops

After the Congress, national level training workshops in "*Handling of Reconstructed 3D Human Tissue Models*" were conducted at NFB, Mumbai, lead by Dr Pellevoisin and supported by Prof. Akbarsha. The two two-day workshops were on *OECD TG 439* – In vitro *testing of skin irritation of chemicals using Reconstructed Human Epidermis (RHE)* and *OECD TG 492* – In vitro *testing of eye irritation of chemicals using Reconstructed Human Corneal Epithelium (RHCE)*. Both workshops included an introduction on alternatives to animal experiments and the *in vitro* tissue model, demonstration of SOP of the respective testing protocols, hands-on practice using control and test samples, MTT assay, ELISA plate reading, data analysis and interpretation, followed by certification. The participants (18 per workshop) were excited about the experience. EPISKIN Academy provided the tissue samples as a generous gift. We thank the staff of NFB, particularly Benedict Mascarenhas, Dr Prathiksha Alag (NFB) and Dr Gaganjyot Kaur (NFB).

The overwhelming success of the National Congress and workshops has paved the way for further deliberations and training in the area of alternative methods for safety testing, thus providing a platform for this very important area of research and taking India forward on the global roadmap towards developing and implementing validated alternative methods.

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Meeting Report

Inauguration of the Centro 3R for the Promotion of 3Rs Principles in Teaching and Research

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The first European interuniversity center dedicated to promoting 3Rs principles in teaching and research was inaugurated in Pisa, Italy on March 14, 2018. The Centro 3R[1] was spearheaded by the Universities of Pisa and Genoa. Membership is open to all Italian universities and agreements for twinning across Europe and other countries are being pursued.

The event was attended by around 90 participants including the press, members of animal welfare associations, pro-life associations, industry, technology hubs, scientists and students. It was officially opened by the Vice Rector of the University of Pisa, Professor **Nicoletta De Francesco** and the Rector of the University of Genoa, Professor **Paolo Comanducci**. They both remarked on the timeliness of the initiative and the relevance of teaching and learning in the context of the 3Rs as well as the role of universities in free learning and research. Nicoletta De Francesco highlighted the University of Pisa's commitment to animal welfare and new technology and invited other universities to join the initiative. Their opening speeches were fol-

[1] http://www.centro3r.it