ing procedures for the use of animals for experimental and educational purposes.

The participants were assigned to five discussion sessions on cellular and lower animal models, *in silico* and systems biology, future technologies, challenges and expectations of industry, and regulation and implementation.

The outcomes were presented in a common session. This was followed by a panel discussion on "Animal Studies in India: Challenges and the road ahead".

Proposals were made to form a consortium for alternatives research in India to support networking of the stake-holders, to organize this meeting annually, and to approach the government to open a center of excellence for alternatives research.

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Meeting report

New Alternative Models for *In Vitro* Toxicology

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On June 21, 2016, a meeting was held on "New alternative models for *in vitro* toxicology studies," chaired by Prof. Giovanna Mazzoleni and Prof. Francesca Caloni and organized by CELLTOX, the Italian Association of *in vitro* Toxicology, in partnership with MISTRAL (Integrated Models for Prevention and Protection in Environmental and Occupational Health) Research Center, University of Brescia, Italy. The meeting was hosted by the Faculty of Medicine and Surgery, University of Brescia. The aim of the meeting was to present new *in vitro* methodologies and their applications in different areas, from veterinary toxicology to neurotoxicology.

Prof. **Francesca Caloni** (DIMEVET, University of Milan), president of CELLTOX, presented a lecture entitled "Predictive models in veterinary toxicology: *in vitro* epithelial barrier." The main concept was the use of *in vitro* 3D epithelial barrier models as predictive tools for toxicological adverse effects of xenobiotics and their bioavailability in animals and humans. In the area of veterinary toxicology it is critical to emphasize the importance of species-specific predictive tools, like the porcine small intestinal epithelial cell line, JPEG-2 (Zagabresky, 2013), the bovine mammary epithelial cells, BME-UV (Al-Bataineh, 2012) and the 3D dog skin equivalent model air-liquid interface (Serra, 2007) to evaluate the absorption, bioavailability, metabolism, and toxicity of natural or synthetic xenobiotics, alone or in mixtures. Moreover, a species-specific bovine primary granulosa cell model was introduced as a predictive tool for endocrine disruptor effects (EATS mechanisms) (Petro, 2012).

Dr **Susanna Alloisio** (ETT, Genoa) presented a sensitive *in vitro* tool useful to detect and evaluate neurotoxicity not only of pure chemicals, such as pesticides or drugs, but also of mixtures of not completely known composition, such as environmental biotoxins and herbal oils. The approach evaluates the spontaneous electrical activity developed by primary neuronal networks derived both from embryonic or neonatal mouse cortex grown on microelectrode array (MEA) chips. The major advantages of this *in vitro* cell-based model are the ability to reproduce to a great extent the functional activity of CNS *in vitro* and the ability of MEAs to automatically record neuronal activity over several days or weeks. Several cultures can be monitored in parallel and followed for days or weeks. The sensitivity, versatility and high throughput make the MEA-based assay useful for the screening of chemicals and mixtures. Furthermore, a specific multiparametric analysis is performed in order to evaluate more accurately the effects of chemicals on neuronal functional activity and their neurotoxic potential (Alloisio, 2015, 2016).

Dr **Marisa Meloni** (Vitroscreen, Milan) presented the topic "Adipocytes: scaffold free microtissues for preclinical and toxicology applications." Her group developed human-adipocyte spheroids. These 3D adipose microtissues

are produced by hanging drop technology starting from suspension of cells of one or more cell types to form scaffold-free spheroids where cells are stimulated to interact with each other and produce extracellular matrix. The spheroids are held in 96-well plates for analysis and characterization at the morphological, biochemical and molecular levels. Different experimental protocols can be defined according to the adipocytes' differentiation and they can be used for up to 21 days after seeding. In particular, the adipose microtissues can be used to assess human adipogenesis/preadipocyte differentiation, human adipocyte lipolysis, metabolism and the modulation effects on adipose cell functions derived from treatment with active ingredients, such as food components and additives.

The different topics addressed by the three speakers covered distinct aspects of the transition from 2D, single cell type, single chemical tests towards the more complex and more physiological tests that are currently emerging in various areas which capture the interaction of different cell types in 3D arrangements to investigate toxic effects of single chemicals or mixtures on multiple endpoints. These developments represent a strong contribution towards the replacement of animal experiments at different stages of the toxicological evaluation of chemicals.

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