Meeting Report

Science and Alternative Methods: Integrated Approaches

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The meeting was held on September 20, 2018 at the University of Insubria, Varese, Italy, organized by the Animal Welfare Body, in collaboration with the Doctoral School. The meeting was chaired by Cristina Giaroni, University of Insubria, and Francesca Caloni, University of Milan, who gave a brief introduction on the role of the Animal Welfare Body and the importance of the implementation of the 3Rs.

Francesca Caloni, with a presentation entitled *Alternative Methods: in vitro approach and research*, introduced the 3Rs concept, with an emphasis on toxicology, and gave an overview that ranged from the description of validated stand-alone non-animal methods to the concept of the integrated testing strategy (ITS) and endocrine disruptor effects. The predictivity of *in vitro* models and the importance of species-specificity were explained (Bertero et al., 2018). 3D models, spheroids, and *in vitro* epithelial barrier models were introduced as promising new tools.

Yula Sambuy, from CREA Research Centre for Food and Nutrition in Rome, presented the state of the art on *in vitro* barrier models of gut and brain. Normal human intestinal cells are particularly difficult to differentiate in culture. The most widely used human intestinal cell culture model is the Caco-2 cell line that, although obtained from a colon adenocarcinoma, forms a monolayer of well differentiated polarized cells, coupled by functional tight junctions, which express several metabolic activities of small intestinal normal absorptive enterocytes (Sambuy et al., 2005). Caco-2 cells are differentiated on permeable filter supports over a period of 2 to 3 weeks. The model is used for studies of transport, metabolism, and acute and chronic toxicity of orally ingested substances, including drugs, contaminants, or diet components. Differentiated Caco-2 cells can be co-cultured with other cell types to study the metabolic interplay among different cell types (Smith et al., 2018). Three-dimensional (3D) organotypic intestinal models (Yu et al., 2014) and intestinal cell models derived from induced pluripotent stem cell lines (iPSC) (Akazawa et al., 2018) are emerging as alternatives to the Caco-2 cell model.

The blood brain barrier (BBB) separates the blood flow in the capillaries from the central nervous system (tissue and extracellular fluid). Different co-culture systems have been proposed to model this complex system. Endothelial cells from the microvasculature are placed in 3D culture with pericytes and/or fibroblasts and astrocytes under a laminar flow that contributes to the formation of specialized endothelial tight junctions typical of the BBB (Wevers et al., 2018). Interesting developments include stem cells (mainly iPSC) co-cultured with astrocytes or pericytes, stimulated to differentiate with a suitable cell culture medium containing a mixture of small molecules, hormones, and growth factors (Aday et al., 2016). These human stem cell-based BBB models still need to be characterized regarding expression and functionality of the transporters that control the physiology of the BBB.

Marisa Meloni, VitroScreen CEO, introduced how cosmetic ingredients are evaluated according to SCCS Notes of Guidance (updated version SCCS/1602/18). Methods were described that have been validated to assess single ingredients or mixtures of few ingredients for skin irritation and corrosion, eye irritation, and skin sensitization potential with reference to the relative integrated approaches to testing and assessment (IATA) published in the last years as OECD Guidance Documents (ENV/JM/MONO(2014)19 for skin corrosion and irritation, ENV/JM/MONO(2017)15 for serious eye damage and eye irritation, and ENV/JM/MONO(2016)29 for skin sensitization). Although, single test methods or integrated strategies (like defined approaches for skin sensitization) can be used to replace animal tests, either on their own or in the context of IATA, the *in vitro* approach has to be defined as a function of the formulation type for the assessment of cosmetic products: biological model, exposure conditions, and end-points to be evaluated should be selected on a case-by-case basis to gain a deeper understanding of the product action on living tissues, while ethically respecting the volunteers.

Ester Papa, University of Insubria, focused her presentation on *in silico* computational chemistry approaches, such as those based on quantitative structure activity relationships (QSARs). These techniques represent a fast and inexpensive way to screen thousands of compounds for their potential adverse effects on humans and the environment on the basis of their molecular structure. Modeling strategies based on QSARs rely on the assumption that biological activities/properties of chemicals are intrinsically dependent on their molecular structure. Different endpoints that are fundamental in the identification of potential hazards and risks associated with chemicals can be predicted starting from QSAR models, such as the PBT properties (i.e., environmental persistence, bioaccumulation, and toxicity), or biotransformation (i.e., a key determinant for bioaccumulation assessment) in aquatic and terrestrial organisms (Papa et al., 2014, 2018).

In their studies, Prof. Papa and colleagues addressed the development, validation, and application of QSARs for the prediction of PBT and biotransformation properties of thousands of chemicals (Papa et al., 2014, 2018). They demonstrated how the combination of multivariate analysis and different modelling ap-

proaches is helpful for refinement of the screening-level assessment of the potential PBT behavior of contaminants of emerging concern, such as pharmaceuticals and personal care products. The refinement was achieved by inclusion of QSAR predictions of the potential biotransformation in multiple organisms in the PBT screening procedure (Papa et al., 2018). These results demonstrate that *in silico* alternatives to animal testing can be efficiently integrated to support more complex frameworks for the hazard and the risk assessment of chemicals. Furthermore, they provide concrete opportunities to identify benign/desirable chemicals and alternatives to undesired chemicals, before and/or after chemical synthesis. Therefore, *in silico* methods can be used to generate priority lists, to guide and focus future experimental tests, and to further reduce the impact on test animals.

At the end of the meeting, an interesting discussion based on the importance of transferring and sharing knowledge and technology in different disciplines, like biomedical research, and the important role of education in the university, involved the whole audience.

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