Meeting report

Toxicology is *IN*: *In Silico, In Vitro, Int*egrated Testing Strategy

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In light of the ever-growing impact of toxicological testing of chemicals as a consequence of recent EU regulations (i.e., REACH, Cosmetics Directive, etc.), toxicology is becoming more and more "IN" and alternative approaches to animal testing in toxicology are increasingly required. A meeting on some of the more recent developments in this field, focused on *in silico*, *in vitro* and integrated testing strategies was organized on December 4, 2015 in Milan by the Italian Association of In Vitro Toxicology, CELLTOX, in collaboration with the Mario Negri Pharmacological Research Institute that hosted the event.

First, an interesting and relatively new *in vitro* model of human hepatocytes, the HepaRG line, was presented by Dr **Montini** of Biopredic, Int., the company that commercialized the cell line. HepaRG (http://www.heparg.com) were obtained by French investigators (Sylvie Rumin, Philippe Gripon and Christiane Guillouzo) from a human cholangiocarcinoma. In culture these cells acquired bi-potent properties and can be driven to differentiate into biliary cells and mature hepatocytes. Differentiated human HepaRG cells express most of the specific liver functions at levels close to those found in primary human hepatocytes, including detoxifying enzymes and drug transporters, thus representing the most differentiated hepatocyte cell line model available so far. These cells have also proven useful to model different liver pathologies, such as cholestasis, steatosis, as well as fibrosis when co-cultured in 3D with stellate cells.

Dr **Aude Kienzler**, EURL/ECVAM Joint Research Centre in Ispra, Italy, presented the activities of the European Union Research Laboratory for Alternatives to Animal Testing (EURL/ECVAM) towards developing and validating new methods, tools and standards in support of EU policies. In particular, the Systems Toxicology Unit works towards the improvement of conventional toxicology performed on animals by promoting next generation safety assessment based on the integration of data deriving from alternative *in vitro* models (cell cultures, organ-on-a-chip, 3D tissues and omic methods), with chemo-informatics, chemical data and *in silico* exposure modelling to obtain a more complete and animal-free safety assessment. This represents an Integrated Approach to Testing and Assessment (IATA) and is strongly based on the weight of evidence approach. IATA represents an approach for hazard identification and characterization and/or safety assessment of chemicals based on multiple information sources, which integrates and weighs all relevant existing data, guiding the targeted generation of new data to inform regulatory decision-making about potential hazards or risks. Such an approach has already been successfully applied in the ECVAM Skin Sensitisation Project and has resulted in the adoption by the Organisation for Economic Co-operation and Development (OECD) of the first two non-animal tests to identify skin sensitizers, the Direct Peptide Reactivity Assay (DPRA) and the KeratinoSens™ (TG 442c and 442d) in February 2015.

Dr **Susanna Alloisio** from the Alternative Toxicity Unit at ETT in Genoa, Italy, presented an interesting new high-throughput electrophysiology assay for neurotoxicity. This assay is based on the formation of neuronal networks on micro-electrode circuits, capable of recording the electrophysiological activity of neuronal cells exposed to different, potentially toxic chemicals. The assay presently utilizes embryonal mouse or rat cortical tissue, a mixed population of neurons, oligodendrocytes, astrocytes and microglia, plated on the microelectrode array (MEA) and forming a neuronal network after 21 days in culture. The neurotoxicity of different chemicals can be assayed in this system at the network level rather than on single cells. In addition, the multi-parametric description of the neuronal networks' activity under the influence of the chemical makes the MEA-based screening platform an accurate and consistent tool for the evaluation of the toxic potential of chemicals. The potential neurotoxicity of many chemicals, including not only pharma drugs but also environmental chemicals, biological contaminants, "smart" drugs, etc., can be tested on this system. In addition, the great sensitivity of the MEA system makes it a potential alternative method to detect and characterize the effects of marine biotoxins. Other advantages of this system are its adaptability to the study of neurodegenerative pathologies, of other types of nervous tissue, even human, alone or in co-culture with other cytotypes, or with cellular models of the blood brain barrier.

Dr **Emilio Benfenati** of the Mario Negri Institute for Pharmacological Research in Milan, which hosted the meeting, presented an update of the newly available tools for an *in silico* approach to toxicity evaluation. These approaches are related to studies done by scientists like Pople and Kohn, Nobel

Prize in 1998, and Levitt, Karplus and Warshel, Nobel Prizes in 2013. The excellent theoretical basis is today combined with some practical, easy tools that can greatly assist the user in the assessment of the properties of chemical substances. Modern *in silico* approaches are not simply black boxes, providing the prediction as only output without any possibility to appreciate the basis of the prediction and its reliability. The *in silico* models are tools that can be used to explore and organize a series of evidence related to the substance of interest, exploring the presence of certain features relevant to the toxicological properties, assisting the user in the comparison with similar compounds (in a read-across perspective), and integrating the prediction with the reasoning and explanation. For example, the platform VEGA (http://www.vega-qsar.eu) integrates batteries of tens of models for a range of properties with an independent software tool that evaluates the applicability domain of each prediction, indicating possible limitations of the models, and showing the most similar compounds useful for read-across. Another program presented at the workshop was ToxRead (http://www.toxread.eu), which provides a global, integrated view of the similar compounds and the possible reasons for toxicity, or lack of toxicity, in a graph that can be navigated.

The meeting was well attended by several young researchers interested in the new developments in the field of alternative toxicological testing and represented a good opportunity for discussion with experts in the field, satisfying some of the aims of CELLTOX.

Francesca Caloni[1], Emilio Benfenati[2] and Yula Sambuy[3]
[1]Università degli Studi di Milano, Department of Health, Animal Science and Food Safety (VESPA), Milan, Italy; [2]Laboratory of Environmental Chemistry and Toxicology, IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy; [3]CREA, Food & Nutrition Research Center, Rome, Italy