

**Dear readers,**

this issue starts with congratulations on the recent 80<sup>th</sup> birthday of our colleague and friend, Franz P. Gruber, and a tribute to his achievements in the field of alternatives.

In his Food for Thought ... contribution, Thomas Hartung calls for a Human Exposome Project. He argues that the technologies needed to achieve this vision of understanding how exposure, next to genetics and pathogens, impacts human health and causes disease are available and can be combined with artificial intelligence to integrate evidence in a way we could only dream of a short time ago.

Alex Cayley et al. describe a network of carcinogenicity adverse outcome pathways (AOPs) derived from the predictive system Derek Nexus by associating 37 AOPs with 60 assays, 351 *in silico* alerts, and data on 13,400 compounds. The authors explore different ways the network can be used and questioned to inform carcinogenicity safety assessments.

The GARD<sup>TM</sup>skin assay was included in OECD TG 442E in 2022 as the first method employing expression of a defined set of genes to predict sensitization potential. Andy Forreryd and colleagues successfully challenge the assay with chemicals that are considered difficult to test, i.e., haptens, hydrophobic substances, and substances of unknown or variable composition, complex reaction products or biological substances (UVCBs).

While the rat is commonly used to assess endocrine disrupting potential, there is uncertainty about how well it predicts such effects in humans. In back-to-back publications, Diana Karwelat and Julia Kühnlenz and colleagues describe a rat and a human thyroid-liver chip for the assessment of both direct and metabolism-dependent thyroid perturbation by environmental chemicals based on two endpoints: modulation of thyroid hormone secretion and its metabolism. They envisage that the models can be used to investigate species-specific differences, to allow quantitative *in vitro* to *in vivo* extrapolation, and thus to reduce and replace *in vivo* testing.

While new approach methodologies (NAMs) to assess the potential cardiotoxicity of pharmaceuticals are well underway, they have not yet been applied to environmental chemicals. Mark Daley et al. explore how these NAMs can be transferred and adapted to the diversity of these chemicals and what biological and regulatory considerations need to be considered in this endeavor.

“Low endotoxin recovery” describes the phenomenon that some pharmaceutical products mask contaminating endotoxins from detection by pyrogen tests, leading to such alerts in bacterial endotoxin tests (BET) triggering confirmation in the rabbit pyrogen test (RPT). Tammy Thurman and colleagues compare endotoxin spike recovery in water or product samples using the BET, RPT, and monocyte-activation test (MAT). They find that the endotoxin spike is masked by some products in all three tests, indicating biological inactivation. Further, they confirm that the BET and MAT detect endotoxin spikes in water with a higher sensitivity than the RPT.

Fiona Murphy and colleagues share the concept developed within the GRACIOUS Framework to group nanoforms for risk assessment using a structured approach. The presented template gathers the relevant information and generates a grouping hypothesis by an integrated approach to testing and assessment (IATA). This approach can improve the efficiency and throughput of risk assessment of nanoforms.

Initiatives to replace fetal bovine serum have also drawn attention to other animal-derived products used in cell culture, especially Matrigel, which is derived from sarcomas grown in mice. Cormac Murphy and colleagues find that different recombinant protein coatings and extracellular matrix derived from fibroblasts can substitute for Matrigel for the maintenance culture of different induced pluripotent stem cell lines (iPSCs) and allow their differentiation to renal podocytes.

In their BenchMarks contribution, Elijah Petersen et al. describe a framework for the quality assessment of a new approach methodology and give examples of artifacts and biases revealed by quality control measurements. The set of tools can help to optimize test reproducibility.

Our events calendar for 2023 is already packed with webinars and meetings, especially the 2<sup>nd</sup> MPS World Summit in Berlin, Germany and the 12<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences in Niagara Falls, Canada. Both are open for abstract submissions!

With best wishes for a healthy and successful 2023,

Sonja von Aulock  
Editor-in-chief