Dear readers,

The new decade is here with innovative technologies, fresh ideas, and exciting opportunities to work together towards our common goal of protecting human health and the environment by developing methodologies that reduce, refine, or, better yet, replace or supersede animal experiments. More than ever we must stay open-minded, creative and informed on developments in related scientific fields so that we may recognize chances and draw together the threads to design novel approaches.

The human exposome is just such a chance for toxicology as laid out in this issue's Food for thought … contribution by Fenna Sillé et al. Machine learning approaches and unfathomable computing power can be harnessed to recognize patterns and associations across populations that will generate new hypotheses about how the sum of exposures over time to chemicals, radiation, microorganisms, and combinations thereof can cause or predispose risk groups to disease.

The article by Stefan Altaner et al. is a specific example of an application of machine learning: The results of three enzyme inhibition assays challenged with a collection of microcystin congeners were used to learn about the toxicity of the many other known structural variants to better predict the danger of toxic algal blooms to human health.

Recognizing that compounds can bind to more than one target in the human body, Elena LoPiparo and colleagues screened the entire proteome, only manageable with cloud computing, to predict all binding partners of the food packaging chemical bisphenol A (BPA) in comparison to the human hormone estrogen. Next to known binding partners, BPA was found to bind a plethora of other proteins, many of which also appear to bind estrogen. This example provides novel insight into the previously underestimated complexity of interactions between compounds and proteins and could be used to predict mechanisms of action and drug side effects.

Adverse outcome pathways (AOPs) are receiving much attention, but as there appears to be some uncertainty about how to add information to them, Taylor Rycroft and colleagues have extensively reviewed AOP resources and developed a streamlined process for linking a chemical to an existing AOP. The process has been documented in an informational online tool termed AOPERA, hoping to encourage more contributors to help expand the library of known AOPs.

A 3D human perfused microvessel-on-a-chip model that can be used to study the adhesion of monocytes to the endothelium as well as markers of inflammation and oxidative stress upon toxic challenge, is introduced by Carine Poussin et al. The model could be used to study mechanisms of atherosclerosis development, for drug discovery or to study the effects of toxic challenges on the vasculature.

Noemi Vanerio et al. use porcine carotid arteries from the abattoir to generate an aneurysm model by crosslinking their collagen to stiffen them. The changes in vessel wall structure and increased distal diameter that develop over 10 days in a pulsatile bioreactor are comparable to aneurysm development in humans, thus the model may lend itself to testing of vascular devices such as stents.

Hannah Schug et al. build on the success of the rainbow trout gill cell line-based assay, which became an ISO guideline test to detect fish acute toxicity for environmental risk assessment in 2019, by assessing the ability of a rainbow trout gut cell line to detect the toxicity of hydrophobic and volatile chemicals, which would be expected to enter the fish in food rather than water. They find an excellent correlation, both with results on the gut cell line and with previous *in vivo* tests in fish, showing that either cell line can predict toxicity of different chemicals robustly and correctly.

Bioengineering tissues is currently limited by the need for an exogenous scaffold. Paninee Chetprayoon and colleagues describe the fast generation of multilayered fibroblast constructs that can be either decellularized to obtain natural extracellular matrix to be repopulated with other cells, or that can be used to patch and heal wounded *in vitro* skin equivalents.

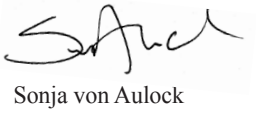
Taking apart a 40-year old assay to identify confounding factors may not seem very sexy to start with, but it can be highly rewarding. Juan Cassano and colleagues meticulously investigate the single cell gel electrophoresis (aka comet) assay to find the source of its high variability. They show convincingly that running the electrophoresis at a constant temperature controlled throughout the tank greatly improves the consistency of the results. This will hopefully improve the acceptance and increase the use of this non-animal method.

Three neuronal models, based on human induced pluripotent stem cells, were challenged with compounds known to induce seizures in humans to investigate whether they could predict this unfavorable property in a drug candidate instead of using animal models or primary animal cells. Anke Tukker and colleagues found that all three models responded with changes in electric activity of the neurons, suggesting that the models could be used to screen drug candidates for this activity or, upon optimization and validation, replace the respective animal experiment.

True to its name, the BenchMarks contribution examines challenges and common pitfalls around the determination of benchmark concentrations. A web-based tool for calculating benchmark concentrations is presented and its usefulness is illustrated with a variety of examples.

Meeting Reports and Corners complement our online News section to bring you up to date with recent developments. Please consult our online Events section to plan your attendance at 3Rs related events this year.

Wishing you a rewarding year,



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