Dear readers,

We hope that this new issue of ALTEX will distract you from coronavirus-related restrictions for a while and remind you that promising developments driven ahead by dedicated scientists are continuing to progress towards the goals of reduction, refinement and replacement of animal experiments.

At long last, monocyte activation tests are causing a drop in the number of rabbits used in pyrogen testing in Europe, and the German Paul-Ehrlich-Institute has announced that it will no longer accept animal data for this endpoint. Thomas Hartung reviews the developments in this field over the past five years, explains the challenges to achieving regulatory acceptance and implementation, and discusses the open issues that still remain 25 years after he published his first paper on the human whole blood assay in ALTEX.

The article by Martin Paparella and colleagues exemplifies the challenges faced by alternative methods when they aim to replace animal tests that were never similarly validated themselves. The authors methodically summarize the limitations and uncertainties of the acute fish toxicity test and argue that environmental extrapolation models combined with alternative methods can provide at least the same level of environmental protection with higher reliability and throughput.

Next to *in vitro* alternatives that have been described and validated for the assessment of skin sensitization potency of chemical compounds, strategies to replace *in vivo* testing include *in silico* models, which predict skin sensitization based on a compound's structure and physicochemical properties. Emily Golden and colleagues compare how well eight such *in silico* models predict two sets of human data on skin sensitization and find that their accuracy is overall comparable to that of the LLNA, a refined animal test, and even better when combined.

Asking on what basis an animal model is selected to address a research question, Désirée Veening-Griffioen et al. examine 110 Dutch project applications for translational and applied research. They conclude that selection is mostly determined by a model and/or related expertise being available rather than whether it has proven predictive value. The need to work with a model of high complexity and intactness often is used as a blanket justification, and 3R statements appear to be used to support the model selection rather than to question it. The authors call on ethical committees and funding bodies to champion change in this area.

The FADU assay is an alternative to the comet assay for the *in vitro* detection of genotoxins, i.e., chemicals that damage DNA. Matthias Mack et al. describe an enzyme-modified automated version of the FADU assay, which can differentiate between different types of DNA lesions. It can be applied both to adherent and suspension cells and thus could also be employed for population studies, e.g., in blood samples, as well as studies on DNA repair.

Cyanobacteria produce liver-toxic microcystins, which are suspected of also being neurotoxic. Stefanie Klima and colleagues investigate this claim by challenging neurons representing the central or the peripheral nervous system with microcystins. They report selective neurotoxicity only at high concentrations unlikely to be achieved upon nutritional or environmental exposure but possible after excessive intake of algae supplements.

In line with the strong trend towards microphysiological 3D *in vitro* models to better represent *in vivo* physiological and pathophysiological processes, Alessandra Marrella et al. present a 3D fluid-dynamic *in vitro* model of ovarian cancer using a cancer cell line supported by an alginate scaffold. They find the efficacy of cisplatin treatment to follow that observed in a mouse model, quite different to that in the static equivalent, and suggest that this model could be useful to predict *in vivo* efficacy of new cancer drugs.

Karsten Mewes et al. report a proficiency exercise showing that the human cell line activation test (h-CLAT) can be used to detect skin sensitizing chemical compounds using antibodies with fluorescent tags that do not interfere with the green autofluorescence of some chemicals including hair dyes, thereby extending the applicability domain of the test.

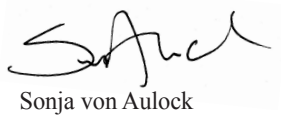
A large database on the measurement of the stress marker corticosterone in mice in scientific papers has been compiled by Stevie van der Mierden and colleagues. In addition, a meta-regression of a part of the data explores how parameters such as the time of day and the method used affect measured endogenous corticosterone concentrations. These outputs can be used to better plan more robust experiments but also to answer research questions in this field by meta-analysis.

John House et al. approach the challenge of characterizing UVCBs (unknown or variable composition, complex reaction products and biological materials), using the example of petroleum substances, based on biological activity signatures derived from highly quality-controlled tests in 15 human cell types. This strategy identifies groups of chemicals of similar biological activity that agree with the hazard potential expected on the basis of their chemical composition. This study impressively demonstrates that such new approach methodologies can be used to group UVCBs, which can allow groups to be characterized based on lead substances and data-gaps to be filled by read-across.

The BenchMarks contribution by Marcel Leist identifies the possible pitfalls associated with working with chemical solutions that are too easily overlooked and may often contribute to failures to reproduce data.

No fewer than seven Meeting Reports testify to the fact that virtual solutions have been found to host scientific symposia despite the Covid-19 pandemic, and the Corners report recent activities of CAAT, Cruelty Free International, EUSAAT, EU-ToxRisk, and LUSH Prize. Please consult https://www.altex.org for upcoming 3R-related events including webinars.

Wishing you a healthy and successful 2021.



Sonja von Aulock
Editor in chief, ALTEX