



Dear readers,

The sad news of the passing of Marty Stephens brings home that while looking forward to the exciting future of the 3Rs field, we should also take time to reflect on its history and the people who have shaped it. This issue contains a report by Michael Balls et al. on the 60 Years of 3Rs symposium with an extensive table documenting the main developments in the 3Rs field by year, and reflections on the many milestones and how they were reached by the people who made it all happen. Inspired by these trailblazing contributions, the Centers for Alternatives to Animal Testing, CAAT and CAAT-Europe, together with ALTEX announce the establishment of the 3Rs Hall of Honor to celebrate the pioneers in the 3Rs field.

In conjunction with DNT-5, this issue's Food for Thought ... contribution by Lena Smirnova and colleagues looks back on the progress in establishing non-animal methods for developmental neurotoxicity testing over the past two decades leading to last year's OECD guidance on the interpretation of the battery of *in vitro* DNT tests. The article also explores the potential of current technological advances to drive the field further toward a better understanding of the hazards of chemical exposure to human brain development.

Aina Mogas Barcons et al. introduce a model of spinal cord injury using slices of spinal cord tissue from chick embryos aiming to partially replace live adult animal testing. Improvements of repair mechanisms in injured sections, such as outgrowth of nerve cells and activity of immune cells, after implantation of a novel scaffold could be demonstrated.

Steven Kunnen et al. analyze transcriptomics gene co-expression networks to identify the mechanisms associated with exposure of primary hepatocytes and HepaRG cells to known liver toxicants and to derive benchmark concentrations. The approach can inform adverse outcome pathways and chemical risk assessment without the use of animals.

Adverse outcome pathways seek to collate and structure information on the toxic effects of chemicals. This concept has been expanded to include non-chemical stressors such as nanomaterials, radiation, viruses, biological therapeutic material, and microorganisms used as pesticides, which can trigger the same key events and adverse outcomes as chemicals. Laure-Alix Clerbaux and colleagues explain the challenges and benefits of accommodating these stressors in the system.

Tom Roos and colleagues describe their protocol to conduct a systematic mapping review of studies on the effects of pollutants on human heart disease that aims to collate a comprehensive database on available evidence for 129 chemicals including heavy metals, air pollutants, and pesticides to contribute to the development of non-animal testing methods.

Calls to plan the phase-out of animal experimentation from governments, parliaments, and non-governmental organizations have brought this discussion into the political arena and incited some ardent opposition. Nico Müller pulls together ten central documents to distill what is meant by the phrase, and what measures, milestones, and monitoring are needed to advance a constructive discussion. The article elegantly contrasts charitable versus uncharitable interpretations of the documents and formulates the central moral argument as well as seven action points.

The t4 Workshop Report by Alexandra Maertens et al. discusses the path towards implementing an artificial intelligence-based probabilistic risk assessment in toxicology. This approach will provide a deeper understanding of the risks of chemicals to human health and the environment.

The t4 Workshop Report by Emily Golden et al. brings forward the discussion on using virtual control groups to replace animal controls in pharmaceutical safety studies by describing an industry initiative to collect and curate existing data, to identify and assess suitable matching criteria to build virtual control groups, and to qualify the approach.

Safety testing seeks to identify any relevant hazard of a chemical on the organism. Walter Zobl et al. challenge the limits of a cell-based test battery representing cells from various sensitive organs by testing a set of chemicals known to cause effects in organs not represented in the battery. Most compounds did cause effects that translated to protective thresholds, and those that did not indicate that the approach can be improved further by adding an assay that captures hematotoxicity.

Meeting Reports, including one on designing a qualification framework for organ-on-chip approaches, and the Corners update you with further developments. The dates for the MPS World Summit, the EUSAAT Congress, and the 13th World Congress as well as other events can be found on the ALTEX website.

Hoping you are inspired by this exciting issue of ALTEX,

Sonja von Aulock
Editor-in-chief