



Dear readers,

Since 2007 we have had one, rarely two Food for Thought ... contributions in each issue of ALTEX (see doi:10.14573/altex.fft for a full list), most of them written or co-authored by Thomas Hartung, director of the Center for Alternatives to Animal Testing (CAAT) in Baltimore, MD, USA and co-director of CAAT-Europe in Konstanz, Germany. From the start, this series has been widely appreciated and highly cited; I have been told that some contributions have inspired the careers of young researchers in the field. Therefore, it is fitting that this issue of ALTEX, which marks the end of our 40th anniversary year, brings you no fewer than four Food for Thought ... contributions. I would like to thank Thomas and all other authors and co-authors for sharing their insightful high-level perspectives on the 3Rs field through this forward-looking format.

In her Food for Thought ... contribution, Katy Taylor asks the simple question, are we making a difference? How are animal use numbers developing in different regions? Is the trend consistently downward? Are biomedical papers without animal experiments on the rise? What have been the drivers of these trends up to now, and what is the path forward?

Thomas Hartung examines the philosophical and ethical concepts relevant to the validation of new approach methods and discusses how these tenets, together with modern scientific technologies and societal expectations, may contribute to reforming the validation process while ensuring human safety and animal welfare.

In a second Food for Thought ... article, Thomas Hartung and colleagues discuss how the validation process can learn from translational medicine, especially biomarkers, for preclinical safety assessment. Biomarkers that are used in the clinic lend themselves as endpoints for new approach methods, and experience with their qualification can feed into NAM validation, making it more predictive and efficient while reducing its reliance on animal testing.

An artificial intelligence-based approach to validation, termed e-validation, can now be envisaged that would optimize the design of validation studies by combining all relevant data sets, selecting reference chemicals, running simulations of the studies, and providing tailored training. This third Food for Thought ... contribution by Thomas Hartung et al. shares a vision of how e-validation may revolutionize toxicological and regulatory science.

To ensure that tetanus vaccines are effective in initiating an immune response, Laura Hassall and colleagues have developed a non-animal method. They now challenge this assay with tetanus vaccine products of different content and quality, show that it can be transferred to other laboratories, and identify suitable reference materials. Once accepted by regulators, the method could replace the animal test currently used for this purpose.

Ceyda Caliskan et al. present a cell-based assay developed to measure the potency of botulinum neurotoxin type A. Using both purified toxin and a commercial product they demonstrate that the assay is as sensitive as the currently used mouse bioassay.

Daniel Ehrlich et al. assemble existing data from high-throughput screening studies to map an adverse outcome pathway linking perturbations of the VEGF signaling pathway to atherosclerosis. They identify several known endocrine disrupting chemicals as relevant stressors of this pathway.

Robert Gutierrez and colleagues assess factors that may influence the results of an *in vitro* oral irritation test that can be applied, e.g., to test the biocompatibility of dental materials in lieu of animal tests.

Virtual control groups, compiled from experimental data on animals used in previous experiments, could replace live control animals in toxicity tests. Andaya et al. identify key parameters that must be matched to those of the experimental groups and use data from an historical experiment to determine whether a virtual control group would have generated the same results. While their results are encouraging, more work is needed to fully realize the concept's potential. In a short communication, Gurjanov et al. focus on the importance of animal body weight as one such key matching parameter in the selection of animals to populate virtual control groups.

Three Meeting Reports and the Corners update you on recent and upcoming activities.

As ever, we are grateful to this year's authors, reviewers, members, sponsors, subscribers and readers for supporting ALTEX.

Sonja von Aulock and the ALTEX Edition Editorial Office with Beatrice Roth and the Board of ALTEX Edition