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It is striking that the 20th edition of the biennial conference of the European Society of Toxicology *In Vitro* (ESTIV) took place in Berlin-Germany almost 3 decades after the breakdown of the Berlin Wall. In fact, since its establishment, ESTIV also has torn down walls around the *in vitro* toxicology silo, and thereby has considerably contributed to the multidisciplinary area this field now has become. Indeed, by synergizing expertise from a diversity of disciplines, including toxicology, cell biology, bio-engineering, chemistry and bio-informatics, several cutting-edge *in vitro* and *in silico* methods have been generated, especially over the last few years, all that actively assist in the implementation of the 3Rs principle in life sciences, in particular in toxicology. Throughout the years, ESTIV has served as a soil and platform for generating and, especially, sharing knowledge regarding novel *in vitro* and *in silico* methods. The 20th edition of its conference, which was organized on 15-18 October 2018 in collaboration with the German Toxicology Society (GT) and the Center for Alternatives to Animal Testing-Europe (CAAT-Europe), was no exception to this reputation. Under the general theme “*new approach methodologies for in vitro toxicology applications*”, the ESTIV2018 conference consisted of 8 thematic sessions focused on bio-engineering and stem cell models, toxicokinetics and *in vitro-in vivo* extrapolation, models, biomarkers and assays for systemic toxicity testing, disease models and translational toxicology, local toxicity and skin sensitization testing, *in silico* modelling and read-across approaches, inhalation toxicity testing and regulatory toxicology. The ESTIV2018 conference program also contained a number of special sessions, including a continuous poster session, 2 student sessions, 2 European project sessions and 2 lunch sessions. The conference was preceded by 2 theoretical workshops, focused on adverse outcome pathways and good *in vitro* method practices/good cell culture practice, and was followed by 1 practical workshop, devoted to *in vitro* skin irritation testing, organ-on-a-chip modelling, *in silico* physiology-based kinetic modelling and quantitative *in vitro-in vivo* extrapolation.
Overall, the ESTIV2018 conference gathered 455 participants originating from 45 countries with an academic, industrial or regulatory background. Besides the science, the program included some social activities, which allowed the conference attendees to meet their colleagues in a more relaxed atmosphere. During the opening ceremony of the conference, ESTIV honorary membership was awarded to Dr. Chandra Eskes, who served the ESTIV Board from 2007 to 2018 and acted as ESTIV President between 2012 and 2016. Furthermore, ESTIV signed a memorandum of understanding with the American Society for Cellular and Computational Toxicology (ASCCT) and the Japanese Society for Alternatives to Animal Testing (JSAAE) in order to join international forces to advance the field of in vitro and in silico toxicology, and of the 3Rs in general.

The current special issue of Toxicology in Vitro is a compilation of 19 peer-reviewed papers related to scientific subjects presented by the authors during the ESTIV2018 conference. The selection of reference and proficiency chemicals is a critical component of method validation and proficiency evaluations. Kolle et al. present a number of cases in which the selection of reference chemicals led to problems in the reproduction of the reference results and demonstration of technical proficiency (Kolle et al., 2019). Kolesnyk and colleagues describe the application of 3R-alternative methods for classification of pesticides for skin irritation/corrosion and eye irritation in Ukraine. They identified a number of limitations to comply with the Globally Harmonized System and provide perspectives on this matter (Kolesnyk et al., 2019). In a study conducted by Tourneix and colleagues, the predictive performance of a defined approach integrating a stacking meta-model incorporating in silico, in chemico and in vitro assays was evaluated using the Cosmetics Europe skin sensitization database. It was found that the defined approach shows a higher degree of concordance as compared to the local lymph node assay for predicting human skin sensitization hazard (Tourneix et al., 2019). Hasan and colleagues present their work on the transcriptional profiling
of reconstructed human epidermis treated with lactic acid and reveal molecular pathways of skin stinging and burning. It was found that lactic acid induces expression of damage-associated genes, neurotrophic factors and chemokines, while compromising barrier function (Hasan et al., 2019). Ilyushina and group have studied various technical grade pesticide products by using an Ames test and a mammalian erythrocyte micronucleus test in vivo. Limitations of the Ames test related to the high cytotoxicity of pesticides against bacterial cells were encountered and therefore it is recommended to use at least 2 methods for reliable evidence of the safe use of pesticides (Ilyushina et al., 2019). Kejlová and team evaluated the safety of food contact paper in an effect-directed approach by using 3 in vitro toxicological bioassays and chemical analyses. By doing so, it was shown that this battery of tests might be useful to predict possible toxic effects of food-related xenobiotics (Kejlová et al., 2019). Egorova and Kaba studied the mechanism of cytotoxicity of an anionic surfactant in vitro, which may be relevant for its use as a stabilizer of silver nanoparticles. Among their findings are an increase in interleukin production and modification of human androgen receptor activity (Egorova and Kaba, 2019). Jeon and group tested the effects of benzalkonium chloride on cell viability, inflammatory responses and oxidative stress of human alveolar epithelial cells cultured in dynamic culture conditions, and noticed significant differences in metabolic activity and other parameters compared to a static culture system (Jeon et al., 2019). Czekala et al. applied high-content screening analysis to primary human bronchial cells and observed reduced activity of flavored E-liquids compared to regular cigarette smoke condensate (Czekala et al., 2019). Méausoone and team demonstrated the use of cultured human bronchial cells in an air-liquid interface system for studying the toxic effects of toluene and byproducts formed during its catalytic oxidative degradation upon repeated exposure (Méausoone et al., 2019). Tollstadius and colleagues investigated the fungicide carbendazim in 2-dimensional and 3-dimensional alveolar cell culture systems, and were able to demonstrate the occurrence of pulmonary toxicity as well
as the importance of the exposure scenario in this process (Tollstadius et al., 2019). Westphal and team challenged cultured rat macrophages with multi-walled carbon nanotubes and various asbestos fibers. They found that multi-walled carbon nanotubes induce stronger migration of inflammatory cells than asbestos or granular particles, but a similar pattern of inflammatory mediators (Westphal et al., 2019). Marquart et al. describe the establishment and testing of a novel set-up based on human small bowel as a suitable tool to investigate the smooth muscle relaxing effects of candidate therapeutics for organophosphorus compound poisoning (Marquart et al., 2019). A bioengineered intestinal *in vitro* model, based on mimicking the natural micro-environment, was developed and characterized by Jochems and colleagues. Cells in this experimental system show *in vivo*-like differentiated hallmarks and respond appropriately to toxic insults. Therefore, this advanced *in vitro* system could be used for drug and nutrient safety and efficacy testing purposes (Jochems et al., 2019). Using a HepaRG cell culture system, Sharanek and group found that pro-inflammatory cytokines, either individually or in mixture, can modulate cholestatic and/or cytotoxic responses to antibiotics (Sharanek et al., 2019). Jiang and team established an improved 3-dimensional rat follicle culture model using alginate hydrogels to observe the function of follicles and oocytes. This *in vitro* setting could be used for studying the molecular mechanisms associated with developmental and reproductive perturbations induced in granulosa cells and/or follicular membrane cell proliferation-related and differentiation-related triggered by environmental toxicants (Zhang et al., 2019). Olayemi Olugbodi and colleagues performed an *in silico* prediction of toxic and pharmacological activities of major phytoconstituents of *Glyphaea brevis*. High anti-oxidant activity and low toxicity were found, which substantiates the use of *Glyphaea brevis* in traditional medicine (Olayemi Olugbodi et al., 2019). Silva and team used cultures of aggressive and invasive glioblastoma cells to mechanistically investigate the specific anti-cancer potential of the cannabinoid agonist WIN55-212-2 (Silva et al., 2019). By performing an *in silico*
analysis, Hu et al. found that several highly conserved microRNAs have a regulatory function in organismal responses to nanoparticles. This suggests a role as biomarkers of nanotoxicity in a wide range of species, including zebrafish (Hu et al., 2019).

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