drug validation. Examples were given of how the drug discovery field exploits this opportunity (e.g., the CiPA initiative) and of the current endeavours to develop and validate new assays in order to increase the predictive power and thus safety testing by developing better guidelines for implementation by regulatory authorities.

Prof. **Stefan Dübel** (Technical University of Braunschweig, Braunschweig, Germany) presented the phage display method as an alternative to completely replace the use of animals for antibody generation. Antibody phage display allows isolation of fully human antibody fragments from recombinant human antibody gene libraries. Despite the existence of this recombinant replacement method, about half a million animals per year are still used for antibody generation. Polyclonal antibodies derived from animal serum are often not defined with respect to the included antibody specificities, and frequently display unwanted off-target reactivity. Whereas therapeutic antibodies are already produced recombinantly due to their superior quality, the use of recombinant antibodies in diagnostics and research is still rare. Apart from sparing animals, higher quality and specificity of recombinant antibodies should motivate the transition to their use for non-therapeutic applications.

Prof. **Veronika von Messling** (Federal Institute for Vaccines and Biomedicines, Berlin, Germany) spoke about the opportunities and perspectives of alternative methods for licensing and batch testing of medicinal products. The authorization of new vaccines and biomedicines still involves animal experiments at different levels. Many licensed products continue to require testing of every batch before its release, which frequently involves *in vivo* assays. When no function-related test is needed and the detection or quantification of a compound is sufficient, simple standard methods like PCR or ELISA can replace the animal test. A good example is the in-house established method "BINACLE" (binding and cleavage) assay which can, for instance, substitute animal use for safety testing of tetanus vaccines and potency testing of botulinum neurotoxins.

Regulatory approval of an alternative to an animal test requires definition of the relevance and regulatory purpose of the method, its validation, test guideline development, and acceptance. Dr **Sebastian Dunst** (German Federal Institute for Risk Assessment- BfR, Berlin, Germany) presented the process of method development and implementation and pointed out pitfalls that can delay implementation. Problems with reproducibility, predictivity, and relevance of a test are often experienced during the validation phase which can take up to 10 years. Former and current newer validation processes were compared and potential future developments to update the validation process were presented.

Risk assessment and toxicological tests are important for the chemical industry to obtain market approval for new substances. Dr **Barbara Birk** (BASF SE, Ludwigshafen, Germany) presented in-house efforts to validate new approaches and establish alternative methods as well as to gather and assess post-validation data of existent techniques. Alternatives for safety assessment are, for instance, based on regulatory and mechanistic studies as well as on compound screening. The importance of collaborating with regulatory bodies, academia, and other chemical and pharmaceutical companies for the success and rapid implementation of a new method was highlighted and exemplified.

We thank the organizers, especially Dorothea Mühe, the speakers and participants for the pleasant time together as well as for the valuable scientific exchanges on alternatives to animal experimentation.

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Meeting Report

Is There an End in Sight for Animal Testing? Can Organ-on-a-Chip Replace Animal Use in Safety Testing with Advanced Human-focused Approaches?

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The 5th LUSH Prize Conference took place on November 16, 2018 at the *Umweltforum*, Berlin, Germany, ahead of the LUSH Prize 2018 award ceremony. **Rob Harrison**, Director of the LUSH Prize and Editor of Ethical Consumer, welcomed the participants and explained that the mission of the LUSH Prize is to bring forward the date when no further product safety testing in animals is necessary. Reflecting this global issue, past and current winners of the LUSH Prize now represent more than 30 countries.

Ilka Maschmeyer (TissUse, Berlin, Germany) acknowledged that although animal testing is banned for cosmetic products, it is still required for drug development. Although the development of a drug takes on average 13.5 years and costs $2.5 billion, 92% of drugs fail in human clinical trials. To change this, systemic, human cell-based models that better reflect human physiology are urgently needed. The organ-on-a-chip field is now progressing in complexity from 2-4-organ combinations to 10 or more organs to

represent a human-on-a-chip. The miniature organs are connected by circulating fluid in a closed system and are established by using tissue explants, iPS-derived cells, and cell lines. A variety of different organ combinations are available to address different scientific questions.

Renate Künast (Green Party, Germany) reminded the audience that we share the world's limited space and resources with animals. Today, we live in the "cage age" as an overwhelming percentage of the bird and mammalian biomass is made up of agricultural animals. Animal free testing is an important step towards moving out of the cage age. Tools to achieve this overall goal are transparency, i.e., identifying products that were produced with or without animal testing; moving the money, i.e., investing in sustainable research and funding programs for the development of alternative methods; changing the structures, i.e., by better networking, supporting 3R societies, and better representation of animals' interests on animal research committees; better laws, i.e., laws on the European level that are more binding and defined than Directive 2010/63; and continued leadership of the EU parliament, which banned animal testing for cosmetic products and is now working towards an international treaty to extend this to a world-wide ban.

Joachim Wiest (Cellasys GmbH, Munich, Germany) spoke on the challenge of analyzing living cells without interfering with them, i.e., by label-free monitoring of respiration, extracellular acidification, and morphology using microsensors to measure, e.g., pH, oxygen, impedance, and temperature changes during long-term exposure. Cellasys has established a "vegan lab" that uses chemically-defined medium instead of FBS and nonanimal alternatives for other animal-derived products.

The round table session with the first speakers included discussion on Germany's potential to drive change in the European Union, the challenges of finding a common language when different disciplines cooperate, the encouraging development that more young scientists are looking for a career in the 3Rs, and the observation that leading with the argument that high-tech, human cell-based methods can better predict the human response is gaining more recognition and moving alternatives out of the niche of animal protection into mainstream science.

Dr **Dan Dongeun Huh** (BIOLines Research Group, University of Pennsylvania, PA, USA) presented micro-engineered physiological vascularized models developed to represent the complex structure and environment of the human body and its functional units. As an example, he showed detail of a "breathing" lung-on-a-chip, in which a vacuum suction system models the physiological breathing motion. Modeling a lung infection by adding bacteria to the air side activates white blood cells to pass through the endothelial layer to kill the bacteria. Further models of "disease"-on-a-chip include modeling the effects of cigarette smoke, including inflammation, oxidative stress, airway remodeling, and ECM deposition, using a smoking machine, as well as airway constriction in asthma and immunotherapy of cancer. A new project will use chips to investigate how microgravity causes immunosuppression in space. A human "blinking" eye-on-a-chip has been developed that keeps the eye model hydrated by an electromechanical actuator spreading tear film over the cells at intervals; this could be a model to investigate dry eye disease. Placenta and cervix also are being developed for questions relating to reproductive biology and medicine. Current challenges include scaling up the throughput by automating production and culture. According to Dr Huh, these new technologies are changing the way US government agencies think about *in vitro* testing applications.

Herman Koëter (National Committee for the Protection of Animals Used for Scientific Purposes (NCad), The Hague, The Netherlands and Orange House Partnership, Brussels, Belgium) reflected on how the approach to alternatives has changed in the past 60 years. Replacing individual animal tests with single alternative tests has not been a highly successful approach with regard to animal use in science. Fundamental political changes are necessary to make a real impact. Therefore, the Netherlands Minister of Agriculture has set the goal to phase out regulatory animal testing in the Netherlands by 2025. This transition to animal-free testing will require innovative science, politics, and communication with society. Cooperation must be sought between the public and private sector, non-governmental organizations and scientific societies, both within and beyond the Netherlands, to follow and combine all promising pathways.

Terry McCann (TJM Consultancy, Kent, UK) pointed out that *in vitro* assays on cancer cells represent an artificial setting that does not *per se* represent human functionality better than animal tests. Precision engineered human cell culture systems that recreate tissue-level functions, such as organ-on-chips, multiple-organs-on-chips, and human-on-chips, are needed to replicate the complex interactions of human tissues. The major challenge in the alternatives field, for which such chips are very promising, is replacing animal testing for systemic, repeated dose, reproductive and developmental toxicity. Chips also promise to model disease processes and could potentially substitute clinical phase I safety testing. For example, neutropenia caused by an anticancer drug candidate in phase I trials, which had not been observed in prior animal tests, would have been predicted by using a bone marrow-on-a-chip. Barriers to the use of chips are of a technical but also often of a regulatory nature. However, major companies are now adopting the chip technology and creating the evidence that will drive the transition towards a new gold standard.

Troy Seidle (Humane Society International, Canada) informed that more than 40 animal tests are still entrenched in guidelines and regulations and more are under development for, e.g., endocrine disruption, nanoparticles, and genetically modified organisms. Numerous replacement approaches are already available and the 1:1 approach has been supplanted by integrated approaches to testing assessments (IATA). However, international implementation of replacement approaches is slow. For example, the chronic toxicity test in dogs as a second species was shown to be redundant in the 1990s but it has taken 20 years for all major markets to delete this test from their requirements. Major markets must be encouraged to coordinate such decisions. HSI is currently also negotiating with countries that have not yet banned animal testing for cosmetic products and supporting their transition towards nonanimal approaches. The Tox21 initiative has led to a paradigm shift, bringing the potential of *in silico* methodology to the foreground and leading to the consolidation of knowledge of human biology into adverse outcome pathways (AOPs), which

identify relevant modes of action that can be used to design targeted testing approaches based on, among others, microphysiological systems and computational models.

The ensuing round table discussion with the speakers included discussion on translating research results to models that companies can use, validating new approach methods based on reliability and relevance instead of by comparing them to animal tests, and the potential role of AOPs in reshaping the validation process and current challenges of organs-on-a-chip technology.

The book "Animal Experimentation: Working Towards a Paradigm Change" was introduced by **Kathrin Herrmann** (CAAT, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA). While animal experiments are hailed to be indispensable to progress in human healthcare, less than 12% of drugs entering human trials after preclinical testing gain market approval. Studies also have reported that 50-90% of preclinical studies are not reproducible. The book, which is aimed at both scientists and laypersons, reviews the current use of animals in science and presents approaches towards achieving change.

After the break-out sessions, **TJ Bozana** (ToxTrack, Inc., Baltimore, MD, USA) explained how cheminformatics technologies are increasing access to big data, i.e., fusing and analyzing large databases. UL Cheminformatics is a suite of predictive models for nine health hazards based on read-across structure activity relationships (RASAR) developed from machine learning approaches. The analysis of large databases on toxicological data showed that six animal toxicity tests have an average sensitivity of 70%, while the RASAR technology reaches an average sensitivity of 89% across the same end-points with a coverage of 100% of all chemicals.

Bianca Marigliani (INMETRO, Federal University of São Paulo, Brazil) called for abstaining from using animal-derived products, such as fetal bovine serum, trypsin, collagen, etc. for nonanimal methods as they cannot be designated as cruelty-free otherwise. Fetal bovine serum (FBS), which is produced from the blood of fetuses upon slaughter of pregnant cows, has an ill-defined composition, may differ from batch to batch, may be contaminated with pathogens, and may influence cellular assays, can be replaced with human serum or with chemically defined medium. Different formulations of chemically defined media should be tried and cells may need to be adapted to a chemically defined medium by a gradual adaptation strategy.

Alison Gray (Afability, UK) stated that millions of animals are still used worldwide to develop antibodies although cruelty-free methods were developed 20 years ago. The phage-display methodology generates an enormous antibody repertoire with a huge molecular diversity at the antibody binding site from natural or synthetic gene fragments. Clonal selection takes place in bacteria. EU-level guidance and recommendations could help to promote the use of animal-free instead of animal-derived antibodies.

Jan van der Valk (3Rs Centre Utrecht, The Netherlands) spoke on fetal bovine serum, which has been a universal, little questioned cell culture supplement for decades. Replacement of the use of FBS is gaining traction as OECD and FDA are now discouraging its use and recommending the use of chemically defined media. The database "FCS-free" informs on suitable chemically defined media for cells and contains relevant confirmatory data.

Carol Treasure (XCellR8, Cheshire, UK) explained that XCellR8 has established animal product-free methods that are accepted by OECD for testing skin and eye irritation and corrosion by replacing all animal-derived products. There are ethical issues around the use of human-derived reagents in some countries and human serum is far more expensive than FBS, however these reagents make up only a small part of the overall testing costs. In her experience, leading with the science and understanding the practical needs of industry is essential to winning companies over to using cruelty-free methods.

Gill Langley (Humane Society International) closed the conference by summing up the key themes as being technology, multidisciplinarity, interfaces, funding, and positive communication and by thanking the speakers and audience.

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Meeting Report

Advanced *In Vitro* Models Analysis

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Microfabrication techniques and tissue engineering have enabled the development of a wide range of 3D cell culture technologies, including multicellular spheroids, organoids, scaffolds, hydrogels, organ-on-a-chip systems, and 3D bioprinting, each with its advantages and disadvantages. 3D models have been penetrating the early drug discovery process, starting from disease modelling to target identification, validation and screening, lead selection, efficacy, and safety assessment.

However, while challenges remain in the standardization of culture and assay protocols in 2D systems, this is even more pronounced in the more complex 3D cell models, which are less accessible to optical imaging. Therefore, improvements in imaging, data acquisition, and analysis tools including chemical sensing are necessary for broad adoption of 3D cell cultures for screening. Furthermore, regulatory authorities have yet to accept data obtained from 3D cell models as a surrogate or at least as a partial substitution for pre-clinical animal testing.

The Competence Centre TEDD (Tissue Engineering for Drug Development and Substance Testing) is a collaborative innovation platform dedicated to 3D cell culture technology and