then compared to a semi-automated process using KNIME, with analogous threshold rules applied, followed by expert intervention for data points for which the automated system failed to generate an expert call.

The semi-automated expert calls, which were completed in 5 days, compared to 3 weeks for the manual approach, agreed with the human expert calls for 93% of the 6956 chemicals based on a conservative approach for all combined classified calls.

As a proof of concept, the complex nuclear receptor binding/activation data was successfully transformed into a purposeful dataset. This methodology can be extended to other MIEs, including enzyme inhibition. MIE datasets created this way have useful applications, including faster structural alerts development in a transparent *in silico* system like Derek Nexus, for insertion in a purposeful database, to support adverse outcome pathway frameworks and for model building.

S-12.
The neurosphere assay for developmental neurotoxicity testing

Marta Barenys[1], Kathrin Gassmann[1], Christine Baksmeier[1], Sabrina Heinz[1], Martin Schmuck[1], Sivaraj Sundaram[1], Maria Teresa Colomina[2], Heike Heuer[1], Ellen Fritsche [1]

[1]IUF - Leibniz Research Institute of Environmental Medicine, Germany
[2]Psychobiology Unit, School of Psychology, ''Rovira i Virgili'' University, Spain

The neurosphere assay is a model for developmental neurotoxicity screening based on human and rat neural progenitor cells. It enables the detection of disturbances in basic processes of brain development, such as proliferation, migration, differentiation and apoptosis, and the distinction of these specific disturbances from general cytotoxicity. Furthermore, the comparison of human and rat data provides useful insights into species differences for toxicodynamics of compounds contributing to human risk assessment of developmental neurotoxicants. A concrete example of application of the neurosphere assay on the risk assessment of epigallocatechin gallate (EGCG) will be presented within the Adverse Outcome Pathways (AOP) framework. By studying the effects of EGCG, we found a relevant molecular initiating event: binding of EGCG to laminin causing the key event of disturbance of β1-integrin function, leading to decreased adhesion and migration of neural progenitor cells. We propose that developmental neurotoxicity can easily be tested in human neural progenitor cells for recognizing potential human developmental neurotoxic compounds, which thus helps to improve regulatory assessment.

S-13.
The concept of an adverse outcome pathway (AOP) applied to regulatory neurotoxicity evaluation

Anna Bal-Price[1] and Magdalini Sachana [2]

[1]European Union Reference Laboratory for Alternative Methods to Animal Testing (EURL-ECVAM), European Commission Joint Research Centre, Ispra, Italy and [2]Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD), Paris, France

To support a paradigm shift in regulatory toxicology testing and risk assessment, the Adverse Outcome Pathway (AOP) concept has recently been applied to different organ toxicity evaluations, including neurotoxicity. AOPs provide a description of causal rationales for qualitative and quantitative predictive modeling of the human adverse outcome that result from chemical triggering of a molecular initiating event, followed by key events at the cellular and organ level for which testing methods, including high-throughput methods, can be developed.

There are a large number of cellular and molecular processes known to be critical to proper brain development, maturation and function. However, comprehensive understanding of pathways leading from chemical exposure to an adverse outcome in the central nervous system is sparse. This has hampered both the judgment of the predictive ability, as well as the regulatory use of in vitro data. Lately, there has been an attempt to organize the available scientific knowledge in the field of toxicity (including neurotoxicity) by creating AOPs and making them publicly available in a knowledge base (https://aopkb.org).

During this talk, an introduction to the AOP concept and the challenges to apply this framework for developmental neurotoxicity (DNT) testing, followed by an example of an AOP specific for DNT entitled *''Binding of antagonist to NMDA receptors during brain development (synaptogenesis) induces impairment of learning and memory abilities''* will be presented.

Moreover, across the range of potential DNT AOPs common key events will be presented, which could help towards the development and/or interpretation of strategies for Integrated Approaches to Testing and Assessment (IATA) in the field of DNT. This potential application of AOPs related to DNT will increase the use of data derived from in vitro assays and the possibility of correctly identifying potential developmental neurotoxicants, even if toxicity is mediated by various pathways.

S-14.
Studying the blood-brain barrier on a microfluidic chip

Marinke W. van der Helm[1], K. Broersen[2], Andries D. van der Meer[3], Jan C. T. Eijkel[1], Albert van den Berg[1] and Loes I. Segerink [1]

[1]BIOS Lab on a Chip group, MESA+ and MIRA Institutes, University of Twente, Enschede, the Netherlands
[2]Nanobiophysics, MESA+ and MIRA Institutes, University of Twente, Enschede, the Netherlands
[3]Applied Stem Cell Technology, MIRA Institute, University of Twente, Enschede, the Netherlands

A realistic model of the blood-brain barrier (BBB) is valuable to perform drug screening experiments and to improve the understanding of the barrier's physiology at normal and pathological conditions. Although the conventional in vitro systems (e.g. Transwell systems) have been used for this, they lack reproducibility and have a static environment. To overcome these disadvantages so called ''organs-on-chips'' have been developed, which use microfluidics and (human) cells to mimic organ function.

An example of the BBB chip is shown in the work of Griep et al., where human cerebral endothelial cells (hCMEC/D3) were cultured in a microfluidic device made of polydimethyl siloxane (PDMS). Recently we improved this model. Two PDMS parts

with microchannels are placed on top of each other, with a porous membrane in between at the intersection serving as scaffold for the cells. hCMEC/D3 cells (kindly provided by INSERM, Paris, France) were cultured in the chip for up to 15 days. With the four integrated electrodes, which did not block view on the intersection, reliable transendothelial electrical resistance measurements were carried out. Additionally, using immunohistochemistry it was shown that the endothelium expressed tight junction proteins, which is an essential characteristic of the BBB.

To further improve the physiological relevance of this promising platform, the cells inside the channels will be cultured under fluid flow. As application, this platform will be used to study the transport of nanocarriers with Alzheimer medication through the BBB. In addition, the clearance of Alzheimer-associated proteins (amyloid β) by the BBB can be examined.

S-15.
It's all about transport - prediction of blood-brain barrier permeation and *in silico* safety assessment

Gerhard F. Ecker

University of Vienna, Department of Pharmaceutical Chemistry, Pharmacoinformatics Research Group, Althanstrasse 14, 1090 Wien, Austria

With an increasing understanding of the function and physiological role of ABC-transporters, their major contribution to bioavailability, brain permeation, and clearance of drug candidates became evident. P-glycoprotein (P-gp), the paradigm transporter in the field, has been discovered more than 30 years ago as being responsible for multiple drug resistance in tumor cells and for low blood-brain barrier permeability of a large range of compounds. Thus, designing in and designing out substrate properties comprises a hot topic and – due to the polyspecificity of the transporter – also a major challenge for medicinal chemists. Recent progress in the structural biology of ABC-transporter paved the way for the application of structure-based design methods. The combination with ligand-based *in silico* methods derived from the Open PHACTS Discovery Platform allowed to identify molecular features driving drug-transporter interaction. This experimental data guided docking approach was also successfully applied to selected neurotransmitter transporters, such as SERT, DAT, and GAT1.

Successful case studies of this integrated modeling approach comprise ligand- and structure-based design studies on inhibitors of P-glycoprotein as well as ligands of neurotransmitter transporters. Furthermore, mining public data bases allowed the creation of predictive classification models for transporters, as well as the prediction of neuro- and hepatotoxicity based on ligand-receptor interaction profiles.

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S-16.
Microscopic evaluation of in vitro neuronal networks to assess synaptic connectivity and synaptotoxicity

Verstraelen P.[1], Detrez J.R.[1], Pintelon I.[1], Nuydens R.[2], Meert T.[2], De Vos W.H.[1] and Timmermans JP.[1]

[1]Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium
[2]Janssen Research & Development, CNS group, Beerse, Belgium

During brain development, orchestrated activation of genetic programs, as well as spontaneous electrical activity guide the correct wiring of neuronal networks. Mature neuronal networks are characterized by the expression of synaptic markers, synchronized electrical activity and the presence of dendritic spines, tiny protrusions from the dendritic shaft that compartmentalize single synapses to ensure optimal regulation of synaptic strength.

We established an *in vitro* model based on primary hippocampal neurons that recapitulates features of mature neuronal networks. We optimized a set of microscopy workflows to quantitatively characterize both morphological (neurite outgrowth, synapse density, dendritic spine density) and functional (spontaneous electrical activity) aspects of the established neuronal networks.

Using calcium imaging, we pinpointed a critical period in which stochastic activity of individual neurons turned into robust, synchronized network activity, indicative of the formation of functional synapses. This synchronization coincided with an increase in neurite outgrowth and synapse density, while dendritic spine density increased mainly after synchronization of the activity, suggesting that the latter enables fine-tuning of synaptic connections. We further showed that synchronized network activity is mediated by the NMDA receptor, and that interference with microtubule stability alters the bursting pattern.

In brief, we have established a robust platform for pharmacological and/or genetic interrogation of synaptic connectivity in *in vitro* neuronal networks. Our approach is easily amenable to upscaling, which makes it an attractive model for high content synaptotoxicity screening.