Analytica Conference, Munich (Germany), June 22nd 2022 - Joint Symposium by the Society of Toxicological and Forensic Chemistry (GTFCh) and the German Chemical Society (GDCh)

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The Analytica Conference was held in parallel to the Analytica trade fair, which took place from June 21st to 24th in Munich, Germany. The Analytica is the world's leading trade fair for laboratory technology, analysis and biotechnology and takes place every two years at the International Congress Center Messe München. This year's fair and conference was present on site again - after the 2020 event was held virtually for the first time due to the Covid-19 Pandemic. The symposium, which was co-organized by Prof. Hans H. Maurer, Homburg/Saar and Dr. Dirk K. Wissenbach, Jena, both Germany, was divided into three sessions. Each of the sessions being accredited by the GTFCh with two credit points for members who are certified Forensic Toxicologists GTFCh, Forensic Chemists GTFCh, Clinical Toxicologists GTFCh or Forensic-Clinical Chemists GTFCh.

After a welcoming address by Prof. Hans H. Maurer, the first part of the symposium titled **'New Aspects of Clinical & Forensic Toxicology'** started with a lecture held by Dr. Sven Baumann, Institute of Legal Medicine, Department of Forensic Toxicology (University Leipzig, Germany). In his presentation titled 'Possibilities and Limitations of Metabolomics Research in Clinical and Forensic Toxicology' he gave an introduction on how metabolomic strategies can present an alternative option to conventional methods, e. g. when detectability of substances is low or further information is needed. Compared to conventional methods, these metabolomic analyses have the advantage of being more time-sensitive and intensive than other '-omics'. At the same time he emphasized that one will always have differences (sometimes statistically significant) between two groups of people. Continuing with the important point that effectiveness of metabolomics depends on pre-analytical sampling factors and standardization of material, collection, storage and handling.

Nonetheless, metabolomics are in principle suitable for clinical and forensic toxicology if applied properly with reliable and robust analytical methods, comprehensible and reproductive data processing tools, and clear identification of potential targets. At the end of his presentation, he showed different studies on GHB or NPS intoxications and screening for urine adulteration where such an approach could prove helpful.

The latter marked the transition to the second presentation of the day entitled **'Progress on Testing for Sample Adulteration'** by PD Dr. Andrea Steuer, Institute of Forensic Medicine (University of Zurich, Switzerland). Given the fact that drug testing from matrices like urine and hair has become well established for different kinds of purposes, adulteration strategies to avoid positive testing for example in abstinence control have evolved in an equal manner. At the beginning of her talk, she presented the different strategies including substitution or dilution with water or artificial urine as well as chemical adulteration by oxidative treatments. Andrea Steuer then presented an overview of currently available parameters for identifying manipulation of samples and gave an outlook on new advances for the detection of sample adulteration. Although methods like measuring creatinine or using integrated sample checks in immunoassay systems are suitable and routinely used to decide if an urine sample is valid or not, they can still

be subject to false positive or false negative results. A new option of detecting sample manipulation would be to directly identify markers of adulteration such as benzisothiazolinone and ethylene glycols. Unfortunately, some of these markers are also detected in authentic urine samples, which suggests that determining reliable markers for non-biological urine samples is a rather difficult task. The new approaches presented in her talk mainly focus on indirect methods such as detection of phenylacetylglutamine, which only occurs in human thus preventing unnoticed substitution with animal urine. At the end of her presentation, she pointed out that many of the newly proposed adulteration markers offer the advantage to be included in the same analytical run as the principle analyte of interest.

The third lecture was held by Prof. Markus R. Meyer, Department of Experimental and Clinical Toxicology (Saarland University in Homburg/Saar, Germany) and was entitled **'Cytotoxicity Testing – a Task also in Clinical & Forensic Toxicology?'**. He gave the answer right up front and explained that in contrast to old drugs like ethanol, whose toxicity and long term effects are well known, this is not the case for 'New Psychoactive Substances' (NPS). It is strongly suspected that they are indeed toxic, but the mechanism of toxicity, symptoms of overdose and effects of chronic use are still subject of further research. He emphasized that, while therapeutic drug candidates are tested for cytotoxicity during the course of their development to put in relation their therapeutic benefit with their potential toxicity, this does not apply for non-therapeutic recreational drugs like NPS. During his presentation, he presented various assays, which can be used to demonstrate effects on viability as well as cytotoxicity and noted that a single viability test on one cell line alone is not sufficient to prove a relevant toxicity. For example, as the choice of cell line is important it is recommended to select a genetically stable, easily maintained, and well-characterized cell line as for example Caco-2, HaCaT or HEK293. Markus Meyer also showed the utilization of high content screening assays (HCSA), which produce multifluorescence imagines of drug-induced changes in cytobiomarkers and therefore allow for the identification of cytotoxicity. He concluded his lecture with emphasizing that finding the best toxicity monitoring strategy is challenging. Although recent studies have mainly investigated single cytotoxicity biomarkers, it would be preferable to use high-content screening assays in order to obtain a better understanding.

Fig. 1. Speakers and Co-Organizers of the first session (left to right): Hans Maurer, Andrea Steuer, Dirk Wissenbach, Markus Meyer, Sven Baumann.

After the lunch break, before progressing to the second part of the conference held under the theme of 'Rules for Mass Spectrometry Applications', representatives of the GDCh (Dr. Martin Wende), GTFCh (PD Dr. Frank T. Peters), and TIAFT (Dr. Marc A. LeBeau) took the stage. They recapped in short presentations the high-quality and interdisciplinary character of the last 13 biennial symposia at the Analytica Conference, which were all organized by Prof. Hans H. Maurer over more than 20 years. The representatives personally as well as in the name of the respective societies thanked him for his constant commitment and his contribution to the ever-present success of the Analytica Conference (see Fig. 4 at the end of this report).

Afterwards Dr. Marc A. LeBeau from the FBI Laboratory in Quantico, Virginia, USA held a presentation on the **'Identification in Forensic Toxicology: A Radical New Concept or simply jumping onto the Bandwagon?'**. He presented a point-based system for forensic toxicology by the means of an American National Standard that has recently been published (ANSI/ASB Std. 113). The document is setting minimum requirements for analyte identification, which allows laboratories to assess if their testing regimen is sufficient to meet or exceed predefined demands based on a point system while taking into account the wide array of analytical techniques and instrumentation. A rating system is used to assign point values to each technique based on its general specificity e.g. ELISA = 1P, GC = 1P or LCMS = 3P allowing comparison of different analytical techniques. After combining all the applied techniques, a total score can eventually be determined. When the latter meets the predefined minimum score an analyte can be declared as identified. Marc LeBeau illustrated this concept with many practical examples of different relevant scenarios within the field of forensic toxicology. What should be considered in any case is that points are only awarded if the employed analytical methods have been validated and the corresponding validation information has previously been used to assign the appropriate point value. Furthermore, identification requires at least one chromatographic method as well as the use of a reference standard or positive control. Besides, each identified analyte in each matrix must meet the minimum point criteria independently. Advantages of introducing identification points as an internationally recognized approach to verify identities of analytes are that an evaluation of overall information is possible due to addition of points when more than one technique is used and that it complements the more traditional combination of analytical techniques that has been accepted for years.

The next speaker was Prof. Michael Vogeser, Institute of Laboratory Medicine (LMU University Hospital Munich, Germany). He discussed **'Rules for MS Applications in Clinical Laboratories'**. Starting with the difference of immunoassays and LC-MS/MS methods in clinical laboratories he pointed out the advantages of the latter in a clinical context and emphasized on the risks associated with use of laboratory developed tests (LDTs), particularly in patient care context. Afterwards, his presentation focused on the aspect that although within the EU there is the In Vitro Diagnostics Regulation (IVDR) as a key legislative requirement, this mainly applies to LDTs in healthcare facilities rather than fully controlled processes in the safety-critical operation of medical laboratories. Continuing that, even when IVDR is a useful contribution to in-house production of calibration and QC materials, it does not regulate in-house laboratory processes but rather devices.

Fig. 2. Speakers and Co-Organizers second session (left to right): Dirk Wissenbach, Marc LeBeau, Juliane Hollender, Hans Maurer, Michael Vogeser.

One approach to ensure safe processes is the ISO Standard 15189, which acts as a widely used standard for management of resources and processes in the clinical laboratory even if it does not address analytical topics in detail or devices including mass spectrometers. He stated that compliance with the 15189-Standard is legally binding in some settings and countries and it is complemented by the IVDR. Since the European Medicines Agency (EMA) and U. S. Food & Drug Administration (FDA) validation standards are not designed for diagnostic applications,

a precise description of MS-based LDTs seems particularly important. Prof. Vogeser concluded his talk with five recommendations, going from 'fundamental' vs. 'variable characteristics' over clear 'pass criteria', through sustained training as well as supervision of lab technicians to system for instrument maintenance and troubleshooting before finishing with continuity management.

The last lecture of the second session was 'Strategies and Rules for applying HRMS in Environmental Sciences' by Prof. Juliane Hollender, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Environmental Chemistry in Dübendorf, Switzerland. She began her talk by pointing out that there are over 350,000 registered chemicals for productive use worldwide and subsequently showed the workflow applied in environmental analytics. Similar to the system presented by the preceding speaker a point based scoring is carried out and HRMS in combination with gas and liquid chromatography is increasingly used within the environmental sciences for many purposes. For target analysis with reference standards, specific requirements for high-resolution mass spectrometry (HRMS) have been initiated in the EU (EC 2002/657/ED and SANTE/12682/2019). For non-target analysis, such as suspect screening, the definition of minimum requirements is subject to current discussion. Afterwards she presented the NORMAN network to the interested audience. The NORMAN network, in which more than 80 organizations are connected, is aiming to improve the validation and harmonization of monitoring tools through collecting data from collaborative trials and is working on providing a more general guideline at a European level. In the past members of NORMAN have researched on screening methods for water, dust, passive samplers and biota. Prof. Hollender concluded that multi-target, suspect and non-target screening with HRMS allows smart and sensitive identification of contaminant mixtures in environmental monitoring and supports retrospective risk assessment. Proper prioritization is very important and communication of the confidence of identification is necessary. In addition, confirmation with reference material or MS/MS spectra libraries is still mandatory in the regulatory context at this point.

The afternoon and third part of the conference was dedicated to the question 'New Psychoactive Substances – Still a Topic in Forensic Research?'. Prof. Simon Brandt, School of Pharmacy (Liverpool John Moores University, UK) started with **'NPS from Synthesis and Analytics to Pharmacology'**.Using (2-aminopropyl)benzo[b]thiophene analogues (APBTs) as a representative example, moving from synthesis to pharmacological evaluation, Prof. Brandt discussed that the differentiation between various NPS isomers can be a challenge. APBT isomers, e. g. 5- and 6-MAPBT, and many others are new mono-amine transporter ligands that are similar to MDMA in both its structure and effects and bind to the 5-HT2A receptor subtype.

Fig. 3. Speakers and Co-Organizers of the third session (left to right): Christophe Stove, Dirk Wissenbach, Simon Brandt, Robert Kronstrand, Hans Maurer.

He pointed out that, despite having shown activity in vitro they interestingly, and above all unexpectedly, lack stimulant effects as APBT isomers and failed to trigger locomotor activity in mice. This suggests psychedelic and entactogenic effects combined with a low abuse potential,

which in turn could prove as a therapeutic approach to be used in drug-assisted psychotherapy and which is worth investigating further. Referring to a quote of Paul Simon, he emphasized that 'One Man's NPS can be Another Man's Medicine' and outlined the close connection between drugs and medicines, which is also reflected in the fact that a certain number of NPS actually derives from potential drug candidates.

The second talk of the afternoon, **'Advances of Receptor Assays as Tools for Pharmacological Characterization and Analytical Screening of NPS'**, was given by Prof. Christophe Stove, Laboratory of Toxicology (University of Ghent, Belgium). His talk was based on the ever-present challenge of detecting NPS uptake with currently available assays. As immunochemical assays only recognize compounds with similar structure, LC-MS/MS techniques need prior structural knowledge and LC-HRMS struggle to detect unknown metabolites at very low concentrations, new analytical approaches are needed. Prof. Stove introduced an alternative first-line screening tool to examine biological matrices for synthetic opioids and SCRAs (synthetic cannabinoid receptor agonists).

The presented bioassay is independent of antibodies or mass spectrometric detection, but based upon biological activity to monitor receptor activation. The assay can be used to identify specimen that are NPS-positive and additionally serve to generate activity profiles of SCRAs acting on CB_1 and CB_2 receptors, of synthetic opioids acting as μ-receptors agonists and finally psychedelic substances affecting the 5-HT_{2A}-receptor. While activity-based characterization allows prioritization and early risk assessment, activity-based detection allows universal screening for SCRAs and synthetic opioids, which proves useful to rule out a relevant presence of NPS in forensic cases or give a confirmation whenever required. Prof. Stove nicely illustrated the principle of the assay, which is based on a functional complementation of a split NanoLuc luciferase. Once receptor activation is triggered by an NPS, both parts of the nanoluciferase are brought into proximity and the luminescence analyzed. In his presentation, he clearly showed the application of the assay in various use cases. The examples ranged from the determination of the activity of new synthetic opioids (e.g. 2-benzylbenzimidazoles), over screening of serum samples for the presence of SCRA to evaluation of machine learning processes to facilitate the labor intensive manual evaluation of the obtained screening data by individuals.

For the final part of the conference Prof. Robert Kronstrand, Department of Forensic Genetics and Forensic Toxicology of the National Board of Forensic Medicine in Linköping, Sweden held a presentation on **'Current Status of Postmortem-Toxicology of NPS'**. He, like Prof. Brandt before, once again emphasized that NPS are analogues to medicines in all major drug classes and have meanwhile become a serious global problem. Although many countries report encounters with NPS, Sweden and the United States appear to be particularly affected, which may also be subject to the good and thorough local police work within these countries.

Based on the data from Sweden, he showed that NPS-related deaths can be clustered in order to identify patterns. A cluster can be said to exist once five deaths have been observed from one single substance within a 12-month-period. Prof. Kronstrand noted that clusters have become less prevalent (e.g. not one cluster has been observed in Sweden since 2018) possibly because of a more diverse market or less potent, respective less toxic, drugs. While NPS from all drug classes can lead to fatal events, high potency opioid-like NPS can be considered the most deadly mainly because of their risk for respiratory depression. He pointed out, that in postmortem toxicology, analysis is challenging because there is only few pharmaceutical information about concentrations in living subjects, receptor binding properties or behavior in animal studies. In this case, knowledge gained from case reports, as exemplarily presented in his lecture, can contribute to a better understanding. In conclusion, he summarized that more research and data is needed in order to fully assess the effects of NPS in postmortem toxicology.

After nine scientifically exciting and informative lectures and discussions, a successful joint symposium ended with the Co-organizers Prof. Hans H. Maurer and Dirk K. Wissenbach thanking both the speakers and the audience.

Fig. 4. Representatives of the GTFCh, GDCh and TIAFT thanking Prof. Maurer for his commitment to the numberous symposia organized for the Analytica Conferences (left to right): Dr. Dirk Wissenbach, PD Dr. Frank Peters, Prof. Hans Maurer, Dr. Marc LeBeau, Dr. Martin Wende.