VIRTUAL/ONLINE SYMPOSIUM:

CURRENT TRENDS IN FORENSIC TOXICOLOGY

MAY 22 – 24, 2018

Remain Current on Critical Issues Facing Forensic Toxicologists Today!

Novel Psychoactive Substances (NPS)… The opioid crisis…. Fentanyl/Carfentanil…. Screening, identification & confirmation…. Workflow simplification. Learn from some of the world's leading Forensic Toxicologists about these critical issues and the various ways in which they are being addressed. This inaugural virtual symposium provides you with ready access to some of today's leading researchers and practitioners without ever having to leave the laboratory.

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2018 Virtual/OnLine Symposium: Current Trends in Forensic Toxicology

Welcome to the first ever Virtual Symposium on Current Trends in Forensic Toxicology that is being hosted for RTI's ForensicED and sponsored by Agilent Technologies. On May 22nd – 24th 2018, hundreds of attendees will be joining us online to learn from leading researchers and practitioners on extremely important Forensic Toxicology issues facing laboratory professionals today.

Why Should You Attend?

- Insights from leading researchers and practitioners spanning 6 different countries on two continents
- Free registration and no travel costs. Learn without leaving the laboratory.
- On-demand access for content review
- Potential for continuing education credit (see registration page for details)
- Accompanying virtual poster session
- Symposium e-book with abstracts, slides, and presentation summary

We are excited to coordinate and present this amazing Symposium to the Forensic Toxicology Community, and we cannot wait to see you there!

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ForensicED is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of more than 5,000 providing research and technical services to governments and businesses in more than 75 countries, RTI brings a global perspective. ForensicED builds on RTI's expertise in forensic science, innovation, technology application, economics, DNA analytics, statistics, program evaluation, public health, and information science.

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Tuesday - May 22nd, 2018

9am ET – 10am ET / 3pm CEST – 4pm CEST	*The Use of QTOF in Forensic Toxicology* Dr Simon Elliott, Director of Global Forensics, Alere Forensics (now Abbott), Malvern, UK
10am ET – 11am ET / 4pm CEST – 5pm CEST	*Evolving Methodologies Amenable to the Analysis of Drugs in Postmortem Specimens* Dong-Liang Lin, Ph.D., Chief Forensic Toxicologist, Institute of Forensic Medicine, Ministry of Justice, New Taipei City, Taiwan Ray H. Liu, Ph.D., Professor Emeritus, Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, Alabama

Wednesday – May 23rd, 2018

9am ET – 10am ET / 3pm CEST – 4pm CEST	*Coping With Requirements in Forensic Toxicology: Combination of Routine Laboratory and Forensic Science* Prof. Dr. Stefan W. Toennes, Institute of Legal Medicine, Frankfurt/Main, Germany
10am ET – 11am ET / 4pm CEST – 5pm CEST	*LC/MS/MS Approaches for Identifying emerging NPS* Dr. Sarah Kerrigan, Professor and Chair, Department of Forensic Science, Sam Houston State University Dr. Madeline Swortwood, Assistant Professor, Department of Forensic Science, Sam Houston State University

Thursday – May 24th, 2018

9am ET – 10am ET / 3pm CEST – 4pm CEST	*In Vitro Biotransformation of New Psychoactive Substances* Prof. Dr. Alexander van Nuijs, Toxicological Centre, University of Antwerp, Belgium

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10am ET – 11am ET / 4pm CEST – 5pm CEST

Screening and Confirmation Strategies in Postmortem Toxicology

Robert Kronstrand, PhD, Toxicologist, National Board of Forensic Medicine, Sweden

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|Tuesday - May 22nd, 2018|

9am ET – 10am ET / 3pm CEST – 4pm CEST

The Use of QTOF in Forensic Toxicology

Dr Simon Elliott, Director of Global Forensics, Alere Forensics (now Abbott), Malvern, UK

Abstract: The use of high resolution mass-spectrometry (e.g. QTOF) has increased significantly in recent years as it provides a significant number of advantages to analytical toxicology. These include the ability to perform general screening with identification of unknown compounds coupled with retrospective re-interrogation of results if new information comes to light. Targeted analysis can also be performed based on accurate mass detection and QTOF has particular advantages in the detection of more challenging analytes such as New Psychoactive Substances (including synthetic cannabinoids and cathinones) and drug glucuronides. The latter enabling longer windows of detection and confirmation of drug use through metabolite identification. QTOF also allows quantitation of compounds. This presentation will discuss the various advantages as well as the important considerations in implementing high resolution mass-spectrometry in forensic toxicology. These include, identification parameters, detection window interpretation, deuterated internal standards and isobaric/isomeric compounds.

Detailed Learning Objectives:

- Understand how QTOF may be used for general screening, including identification of unknown compounds
- Understand how QTOF may be used for new psychoactive substance analysis
- Understand the considerations of implementing QTOF in forensic toxicology

10am ET – 11am ET / 4pm CEST – 5pm CEST

Evolving Methodologies Amenable to the Analysis of Drugs in Postmortem Specimens

Dong-Liang Lin, Ph.D., Chief Forensic Toxicologist, Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, New Taipei City, Taiwan

Ray H. Liu, Ph.D., Professor Emeritus, Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, Alabama, USA; Editor-in-Chief, Forensic Science Review, Vancouver, Washington, USA

Abstract: The organizational structure and function of the Institute of Forensic Medicine in Taiwan is similar to major medical examiner's offices in the US. The Institute serves the entire country of 23 million. During the recent years, the Toxicology Department received postmortem specimens from

slightly over 4000 cases/year. This laboratory has keenly observed advances in the field of forensic toxicology and actively engaged in developing/adopting new methodologies to constantly increase the laboratory's effectiveness. This laboratory's adoptions of evolving methodologies (preliminary screen, sample preparation, and confirmation/quantitation) amenable to the analysis of drugs are the main focus of this presentation. Data derived from the analysis of drugs in urine specimens serve as the basis to illustrate how new approaches were developed/adopted. Merits of new methodologies are emphasized. For example, the newly adopted UHPLC-QTOF/MS approach enables simultaneous screen of all drugs that were included in the database (established in-house), with significantly higher detection rates over LC-IT/MS and GC/MS based methodologies. On the other hand, a LC-QQQ/MS based methodology can confirm and quantitate many more drugs/metabolites (such as opioids, amphetamines, ketamine, benzodiazepines, barbiturates and new psychoactive substances) in a single analytical run without the derivatization step.

Detailed Learning Objectives:

- Appreciate advances of preliminary screen, sample preparation, and confirmation/quantitation methodologies in forensic toxicology during the last two decades
- Familiarize with the merits of UHPLC-QTOF/MS as a preliminary screen methodology in forensic toxicology
- Recognize potential applications of QuEChERS approaches for sample preparation in forensic toxicology
- Realize the merits of LC-QQQ/MS as a confirmation/quantitation methodology in forensic toxicology

|Wednesday – May 23rd, 2018|

9am ET – 10am ET / 3pm CEST – 4pm CEST

Coping With Requirements in Forensic Toxicology: Combination of Routine Laboratory and Forensic Science

Prof. Dr. Stefan W. Toennes, Institute of Legal Medicine, Frankfurt/Main, Germany

Abstract: Forensic toxicology consists of specialized analytic procedures in the first place, but it also needs evidence based toxicologic evaluation. Analytics on a contemporary level requires high sensitivities for the detection of low concentrations of modern medical and abused drugs. This typically means targeted analyses with liquid chromatography coupled to mass spectrometry (LC-MS/MS), which is not available unlimited in most labs. Therefore, to cope with the needs, the typically limited financial resources must be optimized, e.g. by using diversified equipment like GC-MS, GC-MS/MS and time-of-flight mass spectrometry (TOF). In the forensic lab of Frankfurt/Main, Germany, the principal strategy is to use an LC-MS/MS targeted screening in all cases with the extension of untargeted screening and quantification by LC-TOF MS. Tandem mass spectrometry provides sufficient identification, while for a single stage TOF MS from our experience the All-Ions approach may provide identifying fragments, but not at very low concentrations and the huge data file sizes prohibit routine use. In our laboratory the use of a GC-MS/MS in CI mode with two injectors and LTM (low thermal mass) columns substitutes for sensitive and fast LC-MS/MS, e.g. in the applications to determine cannabinoids in serum and EtG in hair.

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However, forensic toxicology does not only consist of analytics, but also of forensic expertise. This requires continuing education of technical staff and toxicologists, but also practical experience, e.g. by participating in or initiating research projects. Data from controlled studies, especially with human subjects, give a personal impression of intra- and inter-individual variations which is essential for forensic toxicological expertise.

For example, data on effects of “new psychoactive substances” are lacking due to their largely unknown toxicological properties. As a first attempt, a controlled study with the rather long known synthetic cannabinoid JWH-018 was performed where 6 subjects received a 2 and 3 mg dose as well as placebo via inhalation of pure substance in a blinded manner. The low doses produced only small effects but the “subjective high” was elevated and deficits in the critical tracking task and divided attention task were significant. Serum pharmacokinetics covered a time range of 12 hours and JWH-018 and 5 of its metabolites exhibited multi-exponential elimination similar to that of THC. The prominent decrease during the first hour after inhalation suggests a marked distribution which could be the basis of prolonged excretion. This could already be deduced from residual JWH-018 and metabolites in serum and urine 3 and 4 weeks later. In the oral fluid samples (OF) obtained one hour or later after inhalation concentrations were similar to serum with a median OF/S ratio of 1.4 (0.05 –554) but with shorter detectability. In urine the parent compound was not detectable, but 13 conjugated metabolites. The predominant metabolite was JWH-018 pentanoic acid with concentrations less than 5 ng/ml, other major metabolites were 5- and 4-HOpentyl-JWH-018, a hydroxy-keto metabolite and JWH-073 butanoic acid. The different excretion of carboxylic acid and hydroxylated metabolites may aid in evaluation of time of use. Further studies e.g. with increased doses are in progress.

In conclusion, to keep up with modern forensic toxicology requirements, diversified analytical equipment must be accompanied by the respective expertise which can be gained by initiating and participating in scientific studies.

Detailed Learning Objectives:

- Know how to cope with typical analytical tasks in forensic toxicology with a combination of LC-MS/MS, GC-MS/MS, GC-MS and LC-TOF MS.
- Know how to perform analysis of cannabinoids in serum and ethyl glucuronide in hair with one GC-MS/MS device.
- Know about metabolites and pharmacokinetics of JWH-018 in serum, oral fluid and urine.

10am ET – 11am ET / 4pm CEST – 5pm CEST

LC/MS/MS Approaches for Identifying emerging NPS

Dr. Sarah Kerrigan, Professor and Chair, Department of Forensic Science, Sam Houston State University

Dr. Madeline Swortwood, Assistant Professor, Department of Forensic Science, Sam Houston State University

Abstract: With the rapid expansion of NPS and the increased presence of LCMSMS in forensic toxicology laboratories, validated analytical methodologies are necessary for screening and quantification of various NPS classes. Extensive development of extraction techniques has allowed for reduction of matrix

effects, while targeting low limits of detection in smaller and smaller sample volumes. Optimization of chromatography has been key for separating isomers, while still maintaining short runtimes. Advanced mass spectrometric acquisition methods have been designed for screening, identifying, and quantifying these NPS in forensic specimens. We discuss analytical techniques for quantification of synthetic cathinones and screening of fentanyl analogs by LC-QTOF. Additionally, we discuss methods for quantification of synthetic opioids by LC-QQQ. Differences in approaches between the two types of technologies are compared and contrasted.

The fully optimized and validated techniques have been applied to analyze forensic toxicology specimens, study drug stability in biological fluids, investigate drug metabolism pathways, understand postmortem redistribution, and identify novel psychoactive substances. High-resolution mass spectra have been particularly key in developing libraries, characterizing unique biomarkers and metabolites, and identifying novel psychoactive substances from their closely related isomers and analogs.

Understanding pharmacology of emerging compounds is highly reliant upon advanced analytical techniques that allow us to characterize their activity and stability in biological samples or identify metabolites in in vitro assays so that we can better interpret toxicological findings in routine specimens.

Detailed Learning Objectives:

- Attendees will be able to understand differences of quantitative assays by LC-QQQ and LC-QTOF.
- Attendees will be able to identify key steps of method development that allow for sensitive methodologies.
- Attendees will be able to appreciate different approaches to mass spectral data acquisition in LCMSMS.

|Thursday – May 24th, 2018|

9am ET – 10am ET / 3pm CEST – 4pm CEST

In Vitro Biotransformation of New Psychoactive Substances

Prof. Dr. Alexander van Nuijs, Toxicological Centre, University of Antwerp, Belgium

Abstract: The use of new psychoactive substances (NPS) may pose a public health threat, because there is little to no scientific evidence of their pharmacokinetics, recommended dose, effects or safety. Furthermore, NPS can be easily acquired through the internet and smart shops where they are sold under various product labels with often misleading information. From a forensic point of view, NPS are very challenging as there is little information available regarding the metabolic fate of these new substances. The detection in various biological fluids is therefore difficult and possible false positives or false negatives may occur. Characterization of the biotransformation of NPS is important in order to identify suitable biomarkers to be used in forensic screening.

This presentation will present in vitro techniques and workflows to elucidate the Phase-I and Phase-II biotransformation of NPS. Experimental setups for incubations with pooled human liver microsomes and cytosol to generate Phase I and Phase II biotransformations will be shown and discussed, including positive and negative controls. Resulting extracts from the incubations are analyzed with liquid

chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Data analysis for identifying biotransformation products with three different data-analysis workflows will be discussed. A suspect screening workflow was developed using an in-house prepared database built from literature data and in silico biotransformation predictions with the Meteor Nexus software (Lhasa Limited). Furthermore, two non-target screening methods were optimized and applied: (i) using the Agilent MassHunter Qualitative software and (ii) using the open-source software MZmine 2.29 for mass spectrometry data processing. The obtained m/z features were further processed and visualized using R software.

Examples of the techniques and workflows will be given for several classes of NPS such as benzodiazepines (cloniprazepam) and synthetic cannabinoids (5-Cl-THJ-018). For 5-Cl-THJ-018, the results obtained through the in vitro experiments are compared with in vivo results (urine from a 5-Cl-THJ-018 user) to confirm the suitability of the in vitro setup.

Detailed Learning Objectives:

- An in vitro experimental design for investigation of biotransformation of compounds
- Examples and comparison of multiple data analysis workflows (both suspect- and non-target screening)
- Elucidation of the metabolic pathway (Phase-I and Phase-II reactions) of several NPS

10am ET – 11am ET / 4pm CEST – 5pm CEST

Screening and Confirmation Strategies in Postmortem Toxicology

Robert Kronstrand, PhD, Toxicologist, National Board of Forensic Medicine, Sweden

Abstract: Systematic toxicological analysis is the pillar of post-mortem forensic toxicology. It includes the detection, identification, and quantitation of a range of substances including gases, metals, anions, volatiles, pesticides, medications, and drugs of abuse. However, most forensic toxicology laboratories use a case-based progression in their analytical strategy that includes the communication with police and the medical examiner. The reason for this is of course cost effectiveness. On the other hand, a good general rule is to have a tier one panel of screening analyses that is always performed and that can detect and exclude a broad range of relevant substances. Confirmatory analyses always include identification and most of the times also quantitation to enable a correct interpretation.

The aim of this lecture is to describe the possibilities that different screening approaches offer and problematize different workflows for screening and confirmation analyses in a forensic laboratory with focus on post mortem toxicology. The lecture mainly covers analytical strategies for medications and drugs of abuse including new psychoactive substances and is built around the experience from working more than 25 years in a large scale forensic laboratory that handles thousands of autopsy cases on a yearly basis.

Detailed Learning Objectives:

- Recognize the pros and cons of different techniques and methodologies
- Evaluate and select appropriate methodology for the analysis of drugs in post-mortem cases
- Design strategies for successful screening and confirmation

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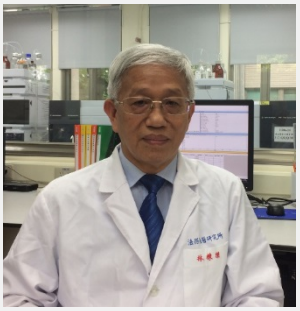
Presenter Bios

Dr. Simon Elliott

Director of Global Forensics, Alere Forensics (now Abbott), Malvern, UK

Dr Simon Elliott has over 20 years' experience in forensic toxicology and is a Consultant Forensic Toxicologist and Director of Global Forensics at Alere (now part of Abbott). He was formerly the founder and Managing Director of Alere Forensics (formerly ROAR Forensics) in Malvern, Worcestershire 2008-2017. He has previously worked as a Clinical Scientist in the NHS at Birmingham City Hospital for over 10 years specifically involved in clinical and forensic toxicology as Section Head of Forensic Toxicology. He is Vice Chair of UKIAFT and an executive Board member of TIAFT.



Dong-Liang Lin, Ph.D.

Chief Forensic Toxicologist, Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, New Taipei City, Taiwan F-ABFT, Former Chief Toxicologist, OCME, Edmonton, Alberta, Canada

Dong-Liang Lin received a Ph.D. degree in pharmacy from Taipei Medical University (Taipei, Taiwan) and postmortem toxicology training in Cook County (Chicago, IL) and New Jersey State (Newark, NJ) Medical Examiner's Offices in US. Dr. Lin joined the Institute of Forensic Medicine (IFM) in 2001, currently serving as the chief toxicologist of the Institute's Forensic Toxicology Department. Prior to joining IFM, Dr. Lin worked (1987–2001) for the Ministry of Justice's Bureau of Investigation laboratory and received training in the US Fish and Wildlife Service Forensics Laboratory (Ashland, OR). Dr. Lin has been actively working on analytical method development and has published more than 40 articles in peer-reviewed journals. Dr. Lin is a member of the American Academy of Forensic Sciences and the International Association of Forensic Toxicologists. He is also a member of the Taiwan Society of Forensic Medicine and the Taiwan Academy of Forensic Sciences.

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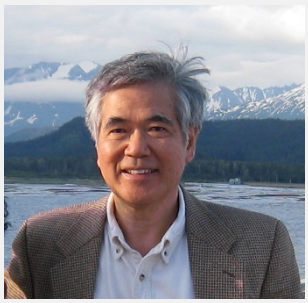
Ray H. Liu



Ph.D., Professor Emeritus, Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, Alabama, USA; Editor-in-Chief, *Forensic Science Review*, Vancouver, Washington, USA

With a law degree from a police academy (now Central Police University) in Taiwan, Ray Liu received a Ph.D. degree in chemistry from Southern Illinois University (Carbondale, IL). Dr. Liu has held positions at the University of Illinois at Chicago (Chicago, IL), US Environmental Protection Agency's Central Regional Laboratory (Chicago, IL), and US Department of Agriculture's Eastern Regional Research Center (Philadelphia, PA) and Southern Regional Research Center (New Orleans, LA). He was a faculty member at the University of Alabama at Birmingham (UAB) for 20 years (serving as the director of the University's Graduate Program in Forensic Science for the last 10 years), retired in 2004, and was granted the "professor emeritus" status in 2005. Following his retirement from UAB, Dr. Liu taught at Fooyin University (Kaohsiung, Taiwan) for eight years (2004–2012). Dr. Liu's work has been mainly in the analytical aspects of drugs of abuse (criminalistics and toxicology), with a significant number of publications in each of the following subject matters: enantiomeric analysis, quantitative determination using isotopic analogs as internal standards, correlation of immunoassay and GC-MS test results, specimen source differentiation, and analytical method development. Dr. Liu authored/edited (or coauthored/coedited) several books, book chapters, and more than 120 articles in refereed journals. He is now the editor-in chief of Forensic Science Review and an editorial board member of several journals.

Prof. Dr. Stefan W. Toennes

Institute of Legal Medicine, Frankfurt/Main, Germany

Stefan Toennes was born in 1966. After studying pharmacy and working in the Institute of Experimental and Clinical Toxicology in Homburg/Saar, Germany, he graduated as Ph.D. in 1997. After habilitation in Forensic Toxicology in 2005 he was appointed extraordinary professor of Goethe University Frankfurt, Germany, and leads its forensic toxicology department. He is member of several scientific committees and currently president of the German Society of Toxicological and Forensic Chemistry.

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Dr. Sarah Kerrigan

Professor and Chair, Department of Forensic Science, Sam Houston State University

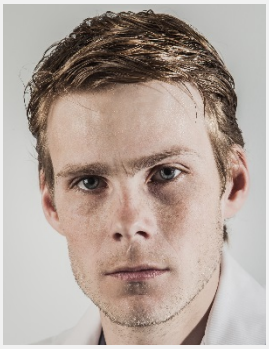
Dr. Kerrigan is Chair of the Department of Forensic Science at Sam Houston State University. She has more than 20 years' experience as a practitioner and researcher in forensic toxicology. She is a former state laboratory director and quality assurance manager.

Dr. Madeline Swortwood

Assistant Professor, Department of Forensic Science, Sam Houston State University

Dr. Swortwood has more than 8 years' research experience with NPS by LCMSMS. She was a former post-doctoral fellow at NIDA and has more than 19 peer-reviewed publications for analytical methodologies and alternative matrices.

Prof. Dr. Alexander van Nuijs



Toxicological Centre, University of Antwerp, Belgium

Alexander van Nuijs is professor in the Department of Pharmaceutical Sciences of the University of Antwerp. His main research area is forensic and analytical toxicology. He has expertise in the analysis of a wide range of analytes (illicit drugs, pharmaceuticals, toxicants) in different matrices (blood, urine, hair, nails, wastewater). One of his main research topics is the investigation of the in vitro biotransformation of new psychoactive substances with the aim to elucidate metabolic pathways.

Robert Kronstrand

PhD, Toxicologist, National Board of Forensic Medicine, Sweden

Dr Kronstrand is the Research Strategist for the National Board of Forensic Medicine (NBFM) in Sweden. He received his PhD in human toxicology in 2001 but already in 1990, he joined the NBFM's department of Forensic Toxicology. He has more than 25 years of experience in postmortem toxicology, DUID, and DFSA and has published more than 70

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papers about forensic and analytical toxicology. He also holds a position as professor in forensic toxicology at the Faculty of Health Sciences, Linkoping University.

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