K1 Effects of Hair Bleachers in the Analysis of Amphetamine(s) and Bath Salt Drugs

Jeffery Hackett, PhD, UCT, Inc, 2731 Bartram Rd, Bristol, PA 19007; Albert A. Elian, MS, and Kerrie Donovan, MS, Massachussetts State Police Crime Laboratory, 59 Horsepond Rd, Sudbury, MA 01776; and Michael J. Telepchak, MBA, UCT, Inc, 2731 Bartram Rd, Bristol, PA 19007*

After attending this presentation, attendees will learn about the effects of hair bleaching in the analysis of amphetamines and synthetic cathinones in hair using readily available Solid Phase Extraction (SPE) cartridges and tandem mass spectrometry.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information about the impact of bleaching materials used on hair analyzed for amphetamines and synthetic cathinones (bath salt) drugs analyzed by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) and solid phase extraction.

Method: Samples of decontaminated hair (10mg) containing amphetamines and bath salt-type drugs (amphetamine, methamaphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone were treated with 10% aqueous sodium hypochlorite solution (bleach), 3% aqueous hydrogen peroxide solution, or 3% aqueous ammonium hydroxide solution for 2 hr before being removed, washed, and dried. The samples were then digested in 0.1M NaOH (containing deuterated analogues) for 0.5 hr at room temperature. Each solution was adjusted to pH6 with 0.1M phosphate buffer (4mL) and applied to a conditioned SPE column. The samples were extracted on commercially available SPE columns (C8/SCX). After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1M), and methanol (3mL of each, respectively). Each SPE column was dried and eluted with 3mL of a solvent consisting of methylene chloride/isopropanol/ammonium hydroxide (78:20:2). After elution, 200µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC/MS/MS in positive Multiple Reaction Monitoring mode (MRM). Data is presented for MRMs of amphetamine, methamphetamine, MDA, and MDMA, butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone (and deuterated analogues), respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1mm C_{18} analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 min and then back to 5% for re-injection. The total run time for each analysis was less than five minutes.

Tandem mass spectrometry was performed in using positive MRM mode. The following transitions were monitored (quantification ions underlined): Amphetamine m/z: 136.1 to 91.0, 65.0, Amphetamine-d_5: m/z 141.1 to 124.0, 93.0, Metamphetamine: m/z 150.1 to 91.1, 119.1 Methamphetamine-d_5: m/z 155.2 to 92.1, 121.2, MDA: m/z 180.2 to 163.1, 105.1, MDA-d_5: m/z 185.2 to 168.1, 110.1, MDMA: m/z 194.2 to 163.1. 105.1. MDMA-d_5: m/z 199.2 to 165.1, 106.8, Butylone: m/z 222.1 to 174.2, 204.2, Ethylone: m/z 222.1 to 174.2, 204.2, Flephedrone: m/z 182.1 to 164.2, 149.1, Mephedrone: m/z 178.1 to 145.1, 160.1, Methylone: m/z 208.1 to 160.1, 132.1, Methedrone: m/z 194.1 to 176.2, 161.1, Methylethylcathinone (4-MEC): m/z 192.1 to 174.2, 144.1, Methylenedioxypyravalerone (MDPV): m/z 276.2 to 135.1, 126.1 and Pyravalerone: m/z 246.2 to 105.1, 175.2, respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of amphetamine and synthetic cathinones

Results: The limits of detection/quantification for the SPE method were determined to be 0.05ng/mg and 0.1ng/mg, respectively for the amphetamines (amphetamine, methamphetamine, MDA, and MDMA) and synthetic cathininones (butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone). The method was found to be linear from 0.1ng/mg to 10ng/mg (r^2>0.999). Data is presented to show that recoveries of amphetamine were found to be greater than 90%. Interday and Intraday analysis of amphetamine were found to <7% and <10%, respectively. Matrix effects were determined to be <5%. Degradation of the amphetamines ranged from 77% to 45%, while the degradation of the synthetic cathinones ranged from 25% to 100% for the bleaching agents.

Conclusion: The use of the information given in this new procedure for the analysis of amphetamine and synthetic cathinones will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC/MS/MS to provide valuable data from about the effects of bleaching agents in hair analysis.

Hair, LC/MS/MS, SPE

 ** Presenting Author*

K2 Extraction and Analysis of AM2201 Metabolites in Urine: A Drugs and Driving Case

Albert A. Elian, MS, Massachusetts Police State Crime Laboratory, 59 Horse Pond Rd, Sudbury, MA 01776; and Jeffery Hackett, PhD, UCT Incorporated, 2731 Bartram Rd, Bristol, PA 19007*

After attending this presentation, attendees will learn about the extraction and analysis of the hydroxy metabolites of AM2201 (a newer fluorinated synthetic cannabinoid) from urine using readily available Solid Phase Extraction (SPE) cartridges and tandem mass spectrometry.

This presentation will impact the forensic science community by offering forensic toxicology analysts and chemists involved with drugs and driving cases more information about the analysis of this synthetic cannabinoid (AM2201 hydroxylated metabolites) employing LC/MS/MS and solid phase extraction.

Method: In this method, urine (1mL samples (calibrators, controls, and test samples)) containing internal standard (THC-d_3/AM2201-OH-d_5) was enzymatically hydrolysed with β-glucuronidase before being adjusted to pH6 with 0.1M phosphate buffer. The samples were then applied to pre-conditioned SPE mixed mode columns (C_8-Weak Anion Exchange). The SPE columns were conditioned with methanol, dionized (DI) water, and 0.1M pH6 phosphate buffer (3mL, 3mL, 1mL, respectively). After loading the samples onto the columns, the SPE sorbents were washed with DI water and pH6 phosphate buffer containing 20% acetonitrile (v/v) (3mL of each) and then SPE columns were dried under full vacuum for 5 min. The analytes were eluted with 3mL of a solvent mixture consisting of ethyl acetate containing 10% methanol. The eluates were evaporated to dryness using nitrogen gas at 40°C and dissolved in 100µL of a mixture of: 95% aqueous formic acid (0.1%) and 5% acetonitrile (containing 0.1% formic acid). The samples were analyzed by tandem mass spectrometry using positive Multiple Reaction Monitoring mode (MRM) and gradient liquid chromatography. Liquid chromatography was performed on a 50x2.0mm C_{18} analytical column with a guard column of the same chemistry. The mobile phase employed consisted of **A** aqueous formic acid (0.1%) and **B** acetonitrile (containing 0.1% formic acid). The gradient was started at 5% **B** and increased 90% **B** in 4 min, after which it was decreased to 5% **B** and kept until 5 min. The flowrate of the mobile phase was 0.5mL per min. Each analytical run was completed in 5 min.

In this presentation, representative chromatograms and calibration curves are shown to illustrate the efficiency of the chromatography and analysis of AM2201 and its metabolites.

Results: The limits of detection/quantification for the SPE method were determined to be 0.5ng/mL and 1.0ng/mL, respectively for the analytes The method was found to be linear from 1.0ng/ mL to 100ng/ mL (r^2>0.999). Data is presented to show that the recoveries of the AM2201 metabolite were found to be greater than 90%. Interday and intraday analysis were found to <7% and <10%, respectively. Matrix effects were determined to be <5%. No parent drug was found in the test sample. Results of the metabolite concentrations are shown in the presentation.

Conclusion: The use of the information given in this new procedure for the analysis of the metabolites AM2201 will be of great use to analysts in the field of forensic toxicology as it demonstrates the use of SPE/LC/MS/MS to provide valuable data regarding the metabolites of one of the newer synthetic cannabinoids

AM2201, SPE, LC/MS/MS

 ** Presenting Author*

K3 Methylenedioxypyrovalerone (MDPV) Postmortem Blood Concentrations: A Series of Suicide Case Reports

George S. Behonick, PhD, Kevin G. Shanks, MS, and Andrea R. Terrell, PhD, AIT Laboratories, 2265 Executive Dr, Indianapolis, IN 46241*

The goal of this presentation is to gain an understanding of the analytical methods employed to qualitatively identify and quantitatively analyze postmortem specimens for the presence of MDPV and other related "bath salt" compounds. Additionally, attendees will gain insight into the interpretive relationships of MDPV postmortem blood concentrations in deaths where drugs were not a factor.

This presentation will impact the forensic science community by emphasizing these unique case histories and circumstances, frequently characterized with atypical or bizarre decedent behaviors, which can direct postmortem toxicological testing strategies to the analyses of MDPV and other "bath salt" compounds.

Methylenedioxypyrovalerone (MDPV), a synthetic beta-ketone resembling methylmethcathinone (mephedrone) and its analogs (commonly termed "bath salts"), has a worldwide distribution and is promoted as a "legal high." Dubbed "not for human consumption," these stimulant agents are easily purchased from a number of sources to include convenience stores, "head shops," and the internet. The agents are identified as "bath salts," "plant food," "jewelry cleaner," and "pipe cleaner" in an apparent attempt to circumvent laws regulating psychostimulant substances. MDPV is a stimulant chemically related to methylphenidate and methylenedioxymethamphetamine (MDMA); its pharmacological mechanism of action is similar to cocaine in acting as a dopamine and norepinephrine re-uptake inhibitor. The products are marketed with clever trade names (e.g., "Ivory Wave," "Vanilla Sky," "Hurricane Charlie," and "Bolivian Bath") and colorful creative packaging. Emergency departments and poison control centers throughout the United States have reported epidemic-like encounters characterized as sympathomimetic toxidromes accompanied by profound mental status and behavioral changes in users. Clinical case reports have also described paranoid psychosis and hallucinatory delirium following the use of MDPV. Persons intoxicated or under the influence of MDPV or other "bath salts" pose keen interest in forensic postmortem and human performance cases; however, routine toxicology screening techniques may not be adequate for detection and identification of these compounds. This study reports an analytical technique for the qualitative and quantitative analyses of MDPV in postmortem specimens. The method is suitable for the analyses of other "bath salt" compounds and is performed routinely by some laboratories in postmortem and human performance cases. The toxicological findings are presented for five postmortem cases submitted to a laboratory between February and November 2011, which include the quantitative analyses of MDPV in blood. The manner of death in all cases was suicide. Three of the cause-of-death classifications involved self-inflicted gunshot wounds, while two cases were attributed to hanging. Three of the five cases were male, with an age range of 25 – 36 years for the five decedents. The range observed for the concentration of MDPV in postmortem blood is 68.3 to 1,044ng/mL. MDPV and other "bath salt" compounds were extracted via a protein precipitation extraction with acetonitrile. Instrumental analysis utilized in identifying and quantifying MDPV was Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Three of five cases exhibited other positive toxicological findings (ethanol, benzodiazepines, THC-COOH, opiates and opioids); one case included detection of mephedrone in blood and other postmortem specimens.

Apparent recreational use of MDPV and other "bath salt" derivatives and analogs is an emerging substance abuse problem. The index of suspicion should be high among forensic pathologists, medical examiners/coroners, and toxicologists when a case history is characterized by bizarre, delusional, and hallucinatory decedent behavior. Profound, intentional self-harm with fatal consequence is described for the five cases in this series of reports; MDPV was quantitatively reported for postmortem blood in all cases, and witness accounts for two of the cases confirmed decedent use of "bath salts" prior to death.

MDPV, Blood, Suicide

 ** Presenting Author*

K4 Fatal Overdose With the Anti-Diarrheal Medication Loperamide

Teresa R. Gray, PhD, 700 N 5th St, Richmond, VA 23219; and Kymberly Carr, BS, 830 Southampton Ave, Ste 400, Norfolk, VA 23510*

After attending this presentation, attendees will be briefed on an apparent suicidal overdose involving loperamide, including analytical parameters used for confirmation and quantitation.

This presentation will impact the forensic science community by describing lethal loperamide concentrations and autopsy observations. This case also highlights the importance of scene investigation and communication between the toxicology laboratory and medical examiners.

Loperamide is a Piperidine Opioid (PO) found in several over-the-counter anti-diarrheal preparations. Doses range from 2 – 4mg PO in adults, not to exceed 16mg daily and expected plasma concentrations are <0.01mg/L. At therapeutic doses, loperamide does not produce typical opioid effects on the central nervous system because of low systemic availability, high protein binding, and poor accumulation in the brain. Loperamide and its primary, inactive metabolite desmethylloperamide are almost immediately pumped out of the brain by P-glycoproteins. Adverse effects are rare, but can include cramps, nausea, drowsiness, dizziness, headache, and dry mouth. Overdoses are usually accidental, nonfatal, and occur in children under age three. Here is reported an apparent suicidal overdose involving loperamide.

A 20-year-old White male with a history of pain and depression was found dead at home along with eight empty 72-count bottles of 2mg loperamide hydrochloride tablets and a receipt of purchase dated one day prior. No suicide note was found. Significant autopsy findings included clear, frothy fluid at the lips and slight vomitus on the face. Biological tissues and fluids collected at autopsy were submitted for toxicological analysis with loperamide noted as a suspected factor in death.

Femoral blood and urine were negative for ethanol, methanol, acetone, and isopropanol. Enzyme-linked immunosorbent assay screening for cocaine metabolite, opiates, methamphetamine/MDMA, phencyclidine, barbiturates, carisoprodol/meprobamate, fentanyl, methadone, zolpidem, amphetamine/phentermine, acetaminophen, and salicylate was negative in femoral blood. HPLC-UV analysis tentatively identified 7-aminoclonazepam, but was not confirmed by mass spectrometry. Alkali-extractable drug screening by liquid-liquid extraction and GC/MS was also negative.

Given the numerous empty bottles on scene and autopsy findings suggesting overdose, it was assumed that loperamide may be present in this case but instrument settings precluded identification as loperamide and desmethylloperamide are reportedly late eluters. As hypothesized, loperamide fortified into drug-free blood, extracted and analyzed by a normal GC/MS screen was not detected. By extending the final hold time, loperamide eluted with substantial tailing. Modifying the temperature ramp further improved peak shape and loperamide was confirmed in femoral blood and urine. Desmethylloperamide was tentatively identified by spectral library match in both matrices, but a reference standard is not commercially available for comparison.

This study successfully quantitated loperamide by GC/NPD using a less polar column and the optimized GC/MS chromatographic conditions. The linear dynamic range was from 0.1 – 6mg/L and accuracy was greater than 85%. Loperamide results obtained in blood, urine, liver, and gastric contents were 0.4mg/L, present, 7mg/kg, and 30mg/kg, respectively; urine was evaluated qualitatively per department policy.

The medical examiner ruled the death a suicidal overdose of loperamide. Two suicidal loperamide overdoses are reported with loperamide concentrations of 2.6mg/L (blood), 12.5mg/kg (liver), and 3mg/kg (gastric contents) in one case and 0.084mg/kg (blood) and 0.87mg/kg (liver) in the other. Blood concentrations in this case and the literature greatly exceed expected therapeutic concentrations. Presumably in overdose, the P-glycoprotein efflux mechanism is overwhelmed, allowing loperamide to exert typical opioid CNS effects leading to euphoria and ultimately death.

Internet-user forums debate the effectiveness of high-dose loperamide to achieve euphoria, ward off withdrawals, and potentiate other opioid receptor agonists. If euphoria is achieved, the high is reportedly not as intense as other prescription opiates/opioids and does not justify the cost or gastrointestinal side effects. Still, since loperamide is easily accessible, the potential for abuse exists and laboratories should evaluate whether their basic drug screens are capable of detecting this late-eluting compound.

Loperamide, Suicide, Death Investigation

 ** Presenting Author*

K5 Analysis of a Group of Volatile Compounds With Forensic Interest: Validation of an Analytical Method by HS-GC/FID

Carla Monteiro, BS, Miguel Franco, MSc, Cristina Cordeiro, MSc, Alda Claro, BS, Paula Proença, BS, Carla Mustra, MS; and Francisco Corte-Real, PhD, Instituto Nacional de Medicina Legal, Largo da Sé Nova, Coimbra, 3000-213, PORTUGAL; and Duarte N. Vieira, PhD, MD, Rua Antonio Jose de Almeida, No 117, Coimbra, 3000-044, PORTUGAL*

After attending this presentation, attendees will understand the potential contribution of a new method for detection and quantification of volatile substances in different biological matrices with interest in forensic contexts.

This presentation will impact the forensic science community by allowing toxicology experts to understand the specificities and difficulties of validating an analytical method developed for the analysis of volatile compounds with different solubilities like toluene or acetone.

Although pharmaceutical products, drugs of abuse and ethanol (alcohol) are the most common poisons encountered in clinical and forensic toxicology, the possibility of poisoning with a wide range of other compounds has to be taken into account. These include pesticides, volatile substances, metals and anions, and natural toxins.

The purpose of this work was the optimization and validation of a sensitive and rapid analytical procedure to the detection and quantification of some volatile organic compounds (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol, chloroform, toluene and xylene) in different matrices (blood, urine and vitreous humor) using a gas chromatograph, equipped with a flame ionization detector coupled to a headspace injector of fixed volume (1mL loop) Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID).

The substances under study were divided and grouped according to their solubility and working range. For substances with high water solubility, a mixture was created (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol). The other substances, whose solubility in water was practically non-existent but had a good solubility in methanol, were divided according to the working range.

Prior to gas chromatographic analysis, all specimens, including the calibrators, were diluted 1:10. By volume, i.e., 100µL of urine, vitreous humor, or blood were diluted with 1mL aqueous solution of n-propanol (100mg/L), used as internal standard.

The chromatographic separation was performed using two capillary columns with different polarities, in order to ensure fulfillment of the identification criteria recommended for this type of analysis (Flanagan *et al*., 1997; Kugelberg *et al*., 2007).[1,2] Chromatographic analysis conditions were as follows: an initial oven temperature of 40°C, held for 5 min, followed by a rise to 130°C with a gradient of 10°C/min. At the end of each analytical cycle, the initial conditions were resumed and maintained for 3 min. The injector temperature was maintained at 150°C, with a split ratio of 4:1, with detectors set at 250°C. The carrier gas was helium at a constant flow rate of 2.7mL/min.

All compounds studied, including n-propanol (internal standard), eluted in a time interval of 15 min and were all well resolved with no interference of metabolites, degradation products, or other substances, such as t-butanol, formaldehyde, ethylene glycol, methyl and ethyl formate, etc. In the concentration ranges analyzed, and for all compounds, the analytical response proved to be linear with a correlation coefficient greater than 0.9962. The limits of detection varied between 1mg/L (1-butanol, toluene, and xylene) and 10mg/L (chloroform) and the limits of quantification between 2mg/L (xylene) and 31mg/L (chloroform). The coefficients of variation obtained for intermediate precision varied from 0.8% (acetonitrile) to 7.0% (xylene). The accuracy of the method varied between 87.8% (acetaldehyde) to 106.3% (xylene).

The study focused on all parameters included in the validation procedure for quantitative methods, in place at the forensic toxicology laboratory of Centre Branch—Portuguese National Institute of Legal Medicine. These included the study of selectivity, linearity, limits of detection and quantification, precision, accuracy, robustness and carryover, having the method shown to be suitable for the intended purpose.

References:

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2. Kugelberg, F.C.; Jones, A.W. (2007), Interpreting results of ethanol analysis in postmortem specimens: a review of the literature, Forensic Science International 165, 10-29.

HS-GC/FID, Volatile, Validation

 ** Presenting Author*

K6 Applications of Hydrophilic-Interaction Chromatography in Forensic Science

Thomas A. Brettell, PhD, and James N. Anasti, BS, Cedar Crest College, 100 College Dr, Allentown, PA 18104*

After attending this presentation, attendees will have a better understanding about hydrophilic interaction liquid chromatography and review of literature displaying how this method can be used for the forensic science community.

This presentation will impact the forensic science community by providing information on the application of Hydrophilic Interaction Liquid Chromatography (HILIC) to the separation of analytes in different matrices including forensic drug and toxicological samples.

HILIC is a mixed or multi-modal partition chromatography designed specifically to separate polar, ionic, or weakly acidic and basic compounds. The aqueous/organic mobile phase is passed over the more polar stationary phase. Columns consist mainly of bare silica or chemically bonded silica such as simple amide, cyano, and diol to complex alkyl and polymeric coatings. The columns can be particle packed or monolithic. The bonded moieties can range in thickness to allow for specific aqueous saturation. The aqueous layer creates electrostatic repulsion and other intermolecular forces to aid in the separation process of more similar compounds such as isomers.

The highly organic mobile phase is composed mainly of acetonitrile and can be controlled through gradient or simple isocratic elution. High concentrations of organic modifiers allow for proper ionization of analytes and, therefore, are compatible with an electrospray ion source of a mass spectrometer. Due to advances in mass spectrometry, this is the detector type of choice when looking at low concentration of analytes and analytes in difficult matrices such as whole blood. As an alternative to normal and reverse phase liquid chromatography, HILIC sustains selectivity and prominent peak shape while using rapid isocratic methods.

Reversed reverse-phase or aqueous normal phase chromatography was coined HILIC by A.J. Alpert in 1990. The first applications of HILIC were primarily of bio analytics such as proteomics and metabolomics because of the ability to purify bio markers, amino acids, and other proteins. In the pharmaceutical industry, the use of HILIC has grown for purposes such as quality control and processes pertaining to research and development. Although HILIC is not a new technique, this form of chromatography is beginning to become more prevalent because of stationary phase developments. Advances in stationary phase preparation, including nanostructures within polymeric scaffold, create efficient preparation and productive permeability. The new production techniques allow not only for a variety of moieties, but also lower cost and create a more consistent product. The separation efficiencies and increased production of HILIC columns has significantly amplified research and applications. Today, HILIC can be seen to be applied to broader applications. HILIC has been applied to many fields, including forensic science and forensic toxicology. Designed for polar metabolites, HILIC is a valuable asset to forensic toxicologists for the analysis of such polar drug metabolites.

Reviews of forensic HILIC applications are seen in such studies as comparison of ethyl-glucuronide distribution in pubic and head hair. Other topics include body fluid and tissue distribution of cocaine and associated metabolites. Estimations of *g*-hydroxybutyrate levels in serum also use HILIC. A method for screening and confirming stimulants, narcotics, and beta-adrenergic agents in urine used the capabilities of HILIC. A comprehensive review for HILIC of seized drugs and related compounds by the Drug Enforcement Agency is also cited. The advantages of HILIC separation of isomers such as morphine-6-glucurinde and morphine-3-glucuronide are also presented. This presentation will review general theory and forensic applications of HILIC for the past 10 years.

HILIC, Forensic, HPLC

 ** Presenting Author*

K7 Analysis and Characterization of the First- and Second-Generation Raving Dragon Novelty Bath Salts

Justin L. Poklis, BS, VCU Dept of PhramTox, 410 N 12th St, Rm 746, PO Box 980613, Richmond, VA 23219-0613; Carl E. Wolf, PhD, Virginia Commonwealth Univ, PO Box 980165, Richmond, VA 23298-0165; Omar I. ElJordi, BS, 3940 Oakleys Ln, Richmond, VA 23223; Kai Liu, PhD, Virginia Commonwealth Univ, PO Box 980581, Richmond, VA 23298-0581; Shinjun Zhang, PhD, Virginia Commonwealth Univ, PO Box 980540, Richmond, VA 23298-0540; and Alphonse Poklis, PhD, VCU/MCVH Station, Medical Coll of Virginia, Box 98-165, Richmond, VA 23298*

After attending this presentation, attendees will see an example of how quickly a single brand of bath salts can change its ingredients as well as the spectroscopic characterization of 3,4-methylenedioxy-N-methylcathinone (methylone) and 2-methylamino-1-phenylpentan-1-one (pentedrone).

This presentation will impact the forensic science community by illustrating an example of the change in active ingredients found in bath salt preparations after the administrator of the Drug Enforcement Administration (DEA) issued a temporary schedule for three synthetic cathinones under the Controlled Substances Act (CSA). These substances were: 4-methyl-N-methylcathinone (mephedrone), 3,4-methylenedioxy-N-methylcathinone (methylone), and 3,4-methylenedioxypyrovalerone (MDPV). Also presented will be the Mass Spectrum, Nuclear Magnetic Resonance (NMR), Ultraviolet (UV), and Infrared (IR) spectroscopic characterization of the first-generation methylone and the second-generation 2-methylamino-1-phenylpentan-1-one (pentedrone), the lone ingredients found in the bath salts sold under the brand "Raving Dragon."

Introduction: In recent years, a large number of new, uncontrolled designer drugs have appeared on the market. Several of the new synthetic drugs that are sold as bath salts belong to one of the classes of β-ketophenylethylamines or phenethylamines. These drugs are available in small packets containing milligrams to gram quantities. They are available via the internet or at various convenience stores, gas stations, truck shops, tattoo parlors, and discount tobacco outlets and are often sold as bath salts with the disclaimer, "Not For Human Consumption."

In February of 2011, several packets containing 0.3g of an off-white powder sold under the name Raving Dragon Novelty Bath Salts were obtained via a website of the same name. This product was removed from the market in October of that year, coinciding with the DEA issuing a temporary schedule of mephedrone, methylone, and MDPV under the CSA. Four months later in February of 2012, a new bath salt was released from the same company under the new name Raving Dragon Voodoo Dust; again several packets were obtained containing 0.5g of a fine white powder.

Methods: Both products were subjected to various spectroscopic techniques: mass spectroscopy (Shimadzu MDGCMS QP-2010 Ultra), NMR spectroscopic (Bruker Ultrashield Plus-400MHz), UV (Shimadzu UV-1601 UV-Visible Spectrophotometer), and IR spectroscopy (Thermo Scientific Nicolet IS10), for the characterization and identification of the active ingredients in the packets. Once the spectroscopic techniques results were obtained for the active ingredients, these results were compared to reference standards in order to confirm their identity and purity.

Results: It was determined that the first-generation Raving Dragon Novelty Bath Salts contained methylone, one of the three compounds added to the banned substance list in October of 2011. The second-generation novelty bath salt, Raving Dragon Voodoo Dust, was found to contain pentedrone. At the present time, pentedrone is unscheduled by the DEA. The purity of the bath salts was determined by UV using the specific absorbance (defined as the $A_{1cm}^{1\%}$ value) of the reference standard vs. the bath salt. Methylone with an $A_{1cm}^{1\%}$ of 550 at λ= 235 was determined to be 89% of the ingredients of the Raving Dragon Novelty Bath Salts. Pentedrone with an $A_{1cm}^{1\%}$ of 579 at λ= 256 was calculated to be 100% of the ingredients of the Raving Dragon Voodoo Dust.

Discussion: Recently, numerous articles relating to the pharmacological and toxicological effects of methylone, including several postmortem cases, have been published. Pentedrone has been previously identified in samples intercepted by the Canada Border Services Agency, customs in Berlin, and police organizations in several federal states of Germany. No specific pharmacological and toxicological data is available.

Conclusion: Once a synthetic compound or group of synthetic compounds are added to the DEA list of scheduled compounds, new analogs appear in their place. In the case of the Raving Dragon Novelty Bath Salts, methylone was replaced within four months of its scheduling with pentedrone as the active ingredient. Pentedrone should be added to the fast-growing group of "Legal High" (designer) drugs that can be expected to be found in bath salt products. The analysis of the Raving Dragon Brand Bath Salts illustrates the rapidly changing active ingredients in "Legal High" preparations that are readily available to the public.

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Bath Salts, Methylone, Pentedrone

 ** Presenting Author*

K8 Characteristics of Toxicology Laboratories Performing Drug-Impaired Driving Casework

Jennifer L. Turri, BS, W 3901 Johnson Rd, Vulcan, MI 49892; Kayla Lowrie, MS, 520 Stevens Dr, Apt 208, King of Prussia, PA 19406; Jillian K. Yeakel, MS, 105 Revere Dr, Harleysville, PA 19438; and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to describe the characteristics of U.S. laboratories performing toxicological analysis in drug-impaired driving cases, focusing on their size, workload, turnaround time, and level of engagement in training. The purpose of presenting this data is to allow attendees to evaluate the findings of a survey of toxicology laboratory users and compare how their own laboratory performs relative to others in terms of size, requests for testimony, and available instrumentation.

This presentation will impact the forensic science community by improving the understanding of expectations and needs of clients of Driving Under the Influence of Drugs (DUID) testing laboratories and standards of service delivery within the field.

Cases involving suspected DUID contain several important elements, including: an officer trained in documenting observations regarding the driving and subject behavior as well as the collection of a biological specimen for comprehensive toxicology testing; a toxicology lab to analyze the specimen for illicit substances; and a prosecutor to utilize the data in the court system. Three surveys were conducted, in collaboration with the National Safety Council, to poll State's Drug Recognition Experts (DREs), Traffic Safety Resource Prosecutors (TSRPs), and toxicology labs with the purpose of gathering information about the needs of the traffic-safety community regarding drug testing and testimony in DUID/DRE cases. TSRPs, DREs, and toxicology lab directors from each state at various jurisdictional levels were surveyed to identify areas of need in the scope and sensitivity of testing available, turnaround time, training, expertise for trial or preparation, meeting court-imposed deadlines, and other service factors of unmet need in training and research for scientists, law enforcement, and prosecutors.

In terms of staffing, the reported mean (median) size of the labs surveyed was 8 (6.5) analysts per lab (range 1-200), with a reported mean (median) of 74 (25) DUID/DRE cases each month per laboratory (range 1-1800). As expected, the size of the lab and the resources available affected the average. In terms of turnaround time, both DREs and TSRPs reported an average turnaround time of eight weeks with respect to drug analysis, and corresponding satisfaction ratings started to decrease among the DREs and TSRPs when turnaround time reached six to eight weeks. According to prosecutors, toxicologist's testimony affects trial outcome a reported average of 63% of the time, and there has been an increase in toxicologist appearances in court due to the confrontation clause issues which in turn contributes to an increase in the analytical backlog in the lab due to analysts being called to testify.

When asked about whether toxicologists are involved in DRE or TRSP training, only 53% of the respondents said they were involved. This type of training entails educating the DREs and prosecutors of what type of testing is provided, specific drugs that are tested for, understanding reports, statistics on drugged driving, and, for prosecutors in particular, how results in a given case are interpreted. When asked why a toxicologist isn't involved in DRE or prosecutor training, the majority reported that they haven't been asked, while others reported that it wasn't seen as necessary or there is insufficient staffing, funds, or resources. In addition to training among the DREs and prosecutors, toxicology labs also reported an additional need for training among the staff. The greatest areas of need for training include instrumentation, uncertainty determination, confirmation testing, and mock-trial training. Other high priorities for additional resources reported by the toxicology laboratories include additional staffing, instrumentation for confirmation, and upgrading or obtaining a new facility.

DUID, Lab Management, Testimony

 ** Presenting Author*

K9 Sertraline in Postmortem Blood and Liver: Deaths in North Carolina (2002 – 2011)

Sandra C. Bishop-Freeman, PhD, 1001 Brinkhous-Bullitt Bldg, Campus Box 7580, Chapel Hill, NC 27599-7580; Ruth E. Winecker, PhD, OCME, Campus Box 7580, Chapel Hill, NC 27599-7580; Laura W. Friederich, BS, 613A Pleasant Dr, Durham, NC 27703; and Erin M. McIntee, MS, OCME, 1001 Brinkhous-Bullitt Bldg, Univ of North Carolina, Campus Box 7580, Chapel Hill, NC 27599*

After attending this presentation, attendees will have greater insight into the different types of postmortem casework associated with sertraline and norsertraline at therapeutic and toxic concentrations.

This presentation will impact the forensic science community by providing information regarding sertraline and norsertraline concentrations as it relates to cause and manner of death determinations.

Sertraline (Zoloft®) is a Selective Serotonin Reuptake Inhibitor (SSRI) used in the treatment for depression in typical, daily adult doses ranging from 50 – 200mg. Patients on chronic oral daily doses of as much as 300mg reached a steady-state plasma level averaging 0.206mg/L (0.099-0.309mg/L). Sertraline undergoes N-demethylation to norsertraline which has about 10% – 20% the pharmacologic activity of its parent.

At the North Carolina Office of the Chief Medical Examiner, cases suspicious for toxicological cause or with essentially negative autopsy findings are routinely screened for common over-the-counter, prescription, and illegal drugs via various laboratory techniques. A search of the database for sertraline/norsertraline liver data with or without corresponding blood data revealed upwards of 200 cases over a 10-year period. Decedents were divided into three groups according to the classification of the effect of sertraline as it impacts the cause of death. The pathologist considered sertraline to be either the primary cause of death (below), additive to the cause of death, or not implicated in death. The foci of the study are the overdose cases where sertraline was determined to be the primary cause of death regardless of other drugs and their concentrations (N=30) and non-overdose cases where sertraline received no classification (N=140). The latter focus may be considered postmortem normal concentrations.

Sertraline metabolism and elimination could be altered by the health of the patient, drug-drug interactions, and genetic deficiencies. The concentrations of sertraline and norsertraline, as well as the parent/metabolite ratio, were reviewed in light of pathological findings and co-intoxicants. Case studies involving significant postmortem redistribution and potential drug interactions will be highlighted.

Sertraline Overdose Cases (Suicide)

Specimen Location	N	Range		Average		Median	
		Sertraline	Norsertraline	Sertraline	Norsertraline	Sertraline	Norsertraline
Central (mg/L)		0.49-25	0.32-8.2	4.19	2.28	1.85	1.5
Peripheral (mg/L)		0.74-9.2	0.27-4.6	2.86	1.79	1.75	1.55
Liver (mg/kg)	20	27-490	5.4-940	137	127	94	72.5

Sertraline Overdose Cases (Accident)

Specimen Location	N	Range		Average		Median	
		Sertraline	Norsertraline	Sertraline	Norsertraline	Sertraline	Norsertraline
Central (mg/L)		0.61-3.1	0.93-4	1.16	1.92	0.82	1.6
Peripheral (mg/L)		0.74-1.8	0.36-3.7	1.21	1.71	1	1.3
Liver (mg/kg)	10	20-279	15-518	90.2	166.8	63.5	155

Sertraline, Death Investigation, Toxicology

 ** Presenting Author*

K10 The Effects of Burn Injury on Tissue Ethanol and Ethyl Glucuronide Concentrations

Trista Haupt Wright, PhD, 700 N 5th St, Richmond, VA; and Kenneth E. Ferslew, PhD, East Tennessee State Univ, Section of Toxicology, Box 70422, Johnson City, TN 37614*

After attending this presentation, attendees will better understand the effects of burn injury on tissue ethanol and ethyl glucuronide concentrations after using a series of burn injury experiments to mimic a residential house fire and determine if visual appearance or core body temperature correlates to changes in analyte concentrations.

This presentation will impact the forensic science community by providing insight to the potential changes in ethanol and Ethyl Glucuronide (EtG) concentrations after burn injury and potential inaccuracies for determining impairment using post-incineration tissue ethanol concentrations.

Ethanol is a popular, legal drug and its deleterious cognitive effects cause an increased risk for residential house fires. Currently, there is no known data available to validate tissue ethanol and EtG concentrations and their interpretations in fire-related death victims. Tissues collected at autopsy must be used for toxicological analysis when blood is not available. The literature does not address the possibility that antemortem tissue ethanol or EtG concentrations maybe altered in fire deaths.

The main objective was to determine if exposure to a house fire causes changes in postmortem ethanol and EtG concentrations from antemortem concentrations.

Methodology included a Sprague Dawley rat model being used to determine the effect of burn injuries, using two fire-related models, on liver, kidney, and heart ethanol and EtG concentrations. The rodents were gavaged with ethanol (4g/kg) then euthanized after three hours by carbon dioxide. Burn injuries from fire deaths were mimicked using the reported average response time by local fire departments and two types of burn injury using a fire pit and a gas grill with these conditions:

Homogenized specimens were analyzed for ethanol by Gas Chromatograph/Flame Ionization Detector (GC/FID) and EtG by enzyme immunoassay. Tissue ethanol and EtG concentrations from burn injury groups, non-ethanol dosed controls, and non-burn injured controls were compared to determine if any differences occurred in analyte concentrations due to flame and/or thermal burn injury. Core body temperatures were monitored using a rectal probe to determine if a correlation existed between changes in analyte concentrations and maximum core body temperatures.

The result was a significant time/temperature increase in tissue ethanol concentrations from both burn injury models. Only the greatest exposure to burn injury with both models produced a significant increase in EtG concentrations. Lesser time/temperature exposures produced a significant decrease in liver ethanol and kidney EtG concentrations. Tissues collected from non-dosed controls did not have detectable ethanol or EtG concentrations produced by burn injury. Changes in ethanol and EtG concentrations and organ weights did not correlate, but changes may be related to maximum core body temperature. Maximum core body temperatures ranged from 96°F – 151°F for burn injury groups.

In conclusion, the burn experiments using a rodent model suggest that caution should be used when predicting ethanol impairment from postmortem fire victim tissue ethanol and EtG concentrations because ethanol and EtG concentrations maybe altered from burn injury. In addition, it was determined that false positives are unlikely in individuals who have not consumed ethanol. This study was unable to determine the mechanism by which the changes in analyte concentrations were altered in corpses exposed to flame or thermal burn injury.

Burn Injury, Ethanol, Ethyl Glucuronide

 ** Presenting Author*

K11 Blood Glucose Concentrations After Burn Injury

Trista Haupt Wright, PhD, 700 N 5th St, Richmond, VA; and Kenneth E. Ferslew, PhD, East Tennessee State Univ, Section of Toxicology, Box 70422, Johnson City, TN 37614*

After attending this presentation, attendees will have a better understanding of burn injury effects on fire victims' blood glucose concentrations.

This presentation will impact the forensic science community by providing an understanding of the potential changes that can occur in post-incineration blood glucose concentrations compared to antemortem blood glucose concentrations in fire-related deaths after burn injury.

Changes in postmortem biochemistry make interpreting toxicology results difficult when attempting to predict antemortem concentrations. Blood glucose concentrations are known to rapidly decrease in the hours following death; however, postmortem vitreous humor glucose concentrations are stable and can be used to determine if hyperglycemia was a factor in the decedent's death. There does not appear to be any literature investigating if burn injury changes antemortem blood glucose concentrations in post-burn injury blood specimens.

The main objective was to determine if post-burn injury blood glucose concentrations are altered by excessive thermal and/or flame burn injury compared to antemortem blood glucose concentrations.

Methodology included a Sprague Dawley rat model being used to determine the effects of burn injuries with two fire-related models, thermal and flame burn injury. The burn injuries produced by the different burn injury groups ranged from scorched hair to loss of limbs. One hundred twenty-six male rats were gavaged orally with 4g/kg of ethanol then placed in metabolic cages for three hours until carbon dioxide euthanization. Burn injuries from fire deaths were mimicked using the reported average response time by local fire departments and two types of burn injury, using a flame and thermal injury, with these conditions:

Pre- and post-burn injury blood glucose concentrations were measured in the heart blood using a Relion Ultima point-of-care blood glucose monitor. Post-burn injury heart blood was collected after refrigeration of the burn injured corpses upon reaching a core body temperature of 50°F. A short duration between death and post-burn injury blood collection and refrigeration was implemented in this procedure to minimize experimental glucose changes. Pre-burn injury core body temperatures and maximum core body temperatures were measured using a rectal probe to determine if there was any correlation between core body temperatures and changes in blood glucose concentrations.

Results revealed maximum core body temperatures ranged from 90°F – 15545°F after flame or thermal burn injury. Post-burn injury blood glucose concentrations in higher maximum core body groups, flame burn injury for eight minutes, and thermal burn injury at 600°F for eight minutes were 30% and 36% greater respectively, compared to pre-burn injury blood glucose concentrations ($p<0.05$). Lesser time/temperature exposures produced a significant decrease in post-burn injury blood glucose concentration ($p<0.05$). Low maximum core body temperature groups had a 50% – 69% decrease in post-burn injury blood glucose concentrations compared to pre-burn injury concentrations. The rate of blood glucose decrease was lessened as time/ temperature exposure increased. Groups that had some of the hotter maximum core body temperatures (thermal burn injury at 400°F for eight minutes, thermal burn injury at 600°F for five minutes, and both burn injury controls) had a much smaller decrease (0% – 27%) in post-burn injury blood glucose concentrations compared to pre-burn injury concentrations.

In conclusion, the burn injury experiments using a rodent model suggest that blood glucose is altered by excessive burn injury. Despite efforts to minimize the loss of blood glucose in post-burn injury, the results indicate that at lower maximum core temperatures, post-burn injury blood glucose concentrations were significantly decreased compared to pre-burn injury concentrations. The natural decline of blood glucose was observed in lower burn injury groups. The decrease in postmortem blood glucose concentrations was disrupted as the burn injury increased in the experiments. The natural process is overshadowed by increasing blood glucose concentrations at higher maximum core body temperatures/longer exposure to burn injury. Blood glucose was significantly elevated in the Sprague Dawley rats that had excessive burn injury/higher maximum core body temperature (thermal burn injury 600°F for 8 min). The literature describes a relationship between hyperglycemia and burn patients. The severely burn-injured corpses exhibited elevated glucose concentrations and suggests that burn injury alters normal postmortem pathological changes. Time and temperature of exposure correlate to core body temperature change and result in a corresponding change in post-burn injury blood glucose concentration.

Blood Glucose, Burn Injury, Rodent Model

 ** Presenting Author*

K12 Evaluation of the Chemical Derivatization of Nine Different Cathinone Bath Salts Analogs

Omar I. ElJordi, BS, 3940 Oakleys Ln, Richmond, VA 23223; Carl E. Wolf, PhD, PO Box 980165, Richmond, VA 23298-0165; Justin L. Poklis, BS, VCU Dept of PhramTox, 410 N 12th St, Rm 746, PO Box 980613, Richmond, VA 23219-0613; and Alphonse Poklis, PhD, VCU/MCVH Station, Box 98-165, Richmond, VA 23298*

The goal of this presentation is to provide the forensic community the optimal derivative to be used in the identification and quantification of bath salts.

This presentation will impact the forensic science community by providing the optimal derivatizing agent, to date, for the drugs cathinone, methcathinone, methylone, methedrone, mephedrone, ethcathinone, ethylone, pentedrone, pentylone, and butylone to utilize in the everyday work of identifying submitted substances. Over the past few years, so-called bath salts drugs have become a major substance-abuse problem in the United States. Cathinone is a naturally occurring stimulant found in *Catha edulis*. It is a beta-keto derivative of amphetamine with similar psychological and behavioral effects. Methcathinone, methedrone, methylone, mephedrone, ethcathinone, ethylone, pentedrone, pentylone, and butylone are designer drugs of cathinone. The beta-keto phenethylamine side-chain varies in length of the alkyl chain at the gamma carbon while the phenyl ring either has no attachment, a methyl group, a methoxy group, or a methylene dioxy ring attached. While cathinone, methcathinone, mephedrone, and methylone are Schedule I drugs, the rest have not yet been controlled. Due to the structure resemblance, highly specific analytical methods such as Mass Spectrometry are necessary to accurately identify these abused bath salts.

A Multidimensional-Gas Chromatography/Mass Spectrometry (MD-GC/MS) (Shimadzu Scientific, Inc.) equipped with a Rtx-5 (20m x 0.18mm ID x 0.2df) and a Rtx-50 (10m x 0.18mm ID x 0.2df) columns (Restek Corporation), a Dean's switch, and a 2010 Ultra GC/MS system with EI ionization was used. The oven temperature was programmed from 150°C, initial hold 0.1 min, to 320°C at 25°C/min. The inlet temperature and transfer temperature were 275°C and 280°C, respectfully. The drugs were initially evaluated underivatized and then derivatized using 5µg of standard in methanolic solution and evaporated to dryness under N_2 at room temperature. The residue was derivatized using either Heptafluorofutyric Anhydride (HFBA), Propionic Anhydride (PA), Acetic Anhydride (AA), Trimethylsilane (TMS, BSTFA + 10% TMCS), t-butyl (MTBSTFA, MTBSTFA + 1% TBDMCS), MethElute, or N-trifluoroacetyl-*l*-triprolyl chloride (*l*-TPC).

The underivatized bath salts analysis resulted in poor chromatographic characteristics and small base peak fragment ions. The optimal derivative was determined to be HFBA, which demonstrated the best chromatographic resolution, characteristic fragmentation, and symmetrical peak shape. HFBA required a lower temperature and shorter reaction time to obtain the derivatives as compared to PA, AA, TMS, and MTBSTFA. Flash derivatization was performed with MethElute. The HFBA derivative results are as follows:

Drug	R_t (min)	Base Ion (m/z)	Qualifier ion m/z (relative abund.%)	Molecular Weight (m/z)
Cathinone	4.5	105	240 (30), 77(4)	345
Methcathinone	4.6	105	254 (29), 77 (81)	359
Ethcathinone	5.0	268	373 (0.2), 134 (2), 105 (50), 77 (21)	373
Mephedrone-D3	5.1	119	257 (21), 91(18)	376
Mephedrone	5.2	119	373 (0.1), 254 (20), 91 (20)	373
Pentedrone	5.2	282	387 (1), 105 (61), 77 (26)	387
Methedrone	5.9	135	254 (8), 107 (6)	389
Methylone	6.4	149	254 (18), 121 (11)	403
Methylone-D3	6.4	149	406 (3), 257(19), 121 (11)	406
Butylone	6.6	149	417 (4), 268 (27), 121 (11)	417
Ethylone	6.7	149	417 (2), 268 (33), 121 (11)	417
Pentylone	6.9	149	431 (3), 282 (21), 121 (10)	431

The shift in retention times of the PA and AA derivatives showed successful derivatization, but the mass spectrum contained uncharacteristic 44m/z and 58m/z base ions. The MTBSTFA, TMS, and MethElute derivatives revealed incomplete derivatization resulting in up to two peaks; a derivatized drug peak and underivatized drug peak or only the completely underivatized. The *l*-TPC derivative was successfully synthesized, resulting in two peaks in mixtures where the solution contained a racemic mixture of the drug.

The HFBA derivative was determined to be the best overall derivative for the identification of the listed bath salts. Bath salts can also be derivatized using *l*-TPC, which can assist in differentiating steroisomers if needed.

Bath Salts, Derivatization, Mass Spectroscopy

 ** Presenting Author*

K13 "Benzofury" Also Known as 6-APB (6-(2- Aminopropyl)-2,3-Dihydrobenzofuran): A Recent Fatality Involving an Unusual Drug

Kimberly McCall-Tackett, BS, Diane J. Mertens-Maxham, BS, Robert E. Lyon, DO, and Norman A. Wade, MS, Maricopa County MEO, Forensic Science Center, 701 W Jefferson St, Phoenix, AZ 85007*

After attending this presentation, attendees will be alerted to a popular new "research chemical" being sold over the internet, 6-(2-aminopropyl)-2,3-dihydrobenzofuran or 6-APB, commonly referred to as "Benzofury" and being used as an ecstasy substitute. This presentation will aid forensic toxicologists in the identification of this unusual compound and provide quantitation data from a recent postmortem case.

This presentation will impact the forensic science community by providing scientific literature from a recent postmortem case involving 6-APB. There is little to no toxicological data available at this time and the presentation of this case may help in compiling such data.

Benzofury (6-(2-Aminopropyl)-2,3-dihydrobenzofuran) or 6-APB, has become a popular "research chemical" available for sale over the internet. It is being marketed as "plant food" and has recently become available in its pure powder form. It is gaining popularity among recreational users for its reported euphoric and psychedelic properties, which are thought to be similar to Methylenedioxyamphetamine (MDA). It is in the phenethylamine class of drugs and an analog of 3,4- MDA, with an oxygen atom within the methylenedioxy portion of MDA being replaced with a methylene group. It is thought to be an entactogen, stimulant, and psychedelic drug and is currently unscheduled in the United States. However, it may be covered in the Federal Analog Act and is still currently legal in several other countries. Dr. David Nichols and his team first investigated 6-APB around 1993 at Purdue as a potential antidepressant which could also possibly be used in conjunction with psychotherapy.

A 21-year-old male had been drinking and using drugs over the course of an evening with two friends at a local motel in Peoria, AZ. They purchased nine pills of what they thought were ecstasy, each consuming three of the pills. During the night, the decedent became aggressive and violent and the friends feared they would be removed from the property. One of the friends put the decedent face down on the bed and straddled him in an effort to calm him. The attempt to restrain him lasted approximately 15 minutes and ended with the friend putting the decedent in a choke hold. The decedent was then found to be unresponsive and emergency medical services were summoned. He was transported to a local hospital where his death was pronounced in the emergency department.

The decedent was transported to the medical examiner's office where a full autopsy was performed. The only notable findings were contusions from the restraint. Multiple postmortem samples including vitreous, cardiac blood, urine, bile, and gastric contents were collected and sent to the toxicology laboratory for testing. Blood and urine specimens were subjected to a qualitative analysis using a basic pH screen with a liquid/liquid extraction and analyzed by Gas Chromatography/Nitrogen Phosphorus Detection (GC/NPD), then confirmed by Gas Chromatography/Mass Spectrometry (GC/MS). Volatiles were assayed on vitreous and cardiac blood using Gas Chromatograph/Flame Ionization Detector (GC/FID). The blood was also screened by Enzyme-Linked Immuno-Sorbent Assay (ELISA) for barbiturates, benzodiazepines, benzoylecgonine, opiates, methamphetamine, and fentanyl. The methamphetamine screen reacted at a low positive level. A sympathomimetic amine quantitation was performed on the cardiac blood by GC/MS Selected Ion Monitoring (SIM), with a large unidentified peak seen on the Thermal Imaging Camera (TIC). A significant peak was also seen on the GC/NPD screen as well as the GC/MS/TIC, which also was unidentified. The peak was subsequently identified as 6-APB and a known standard was obtained courtesy of the DEA Special Testing Laboratory. The 6-APB was quantitated using a sympathomimetic amine method and the concentration was determined by comparing the peak area ratios of 6-APB to the internal standard (MDA-D5) against a standard curve, with linearity demonstrated up to 1.0mg/L. Fractional volumes were used for samples exceeding linearity. The concentration of 6-APB in the decedent's cardiac blood was found to be 2.15mg/L. Ethanol was found in the decedent's cardiac blood and vitreous at 0.05mg% and 0.09 mg%, respectively.

The cause of death was listed as external compression of the neck and the manner of death was homicide. Benzofury (6-APB) was listed a contributing factor. In its pure powder form, 6-APB is usually ingested orally with the onset of effects reported within 30 – 90 min after ingestion. Scant information regarding this drug is available; therefore, little is known about its dosing and toxicity.

Benzofury, MDA, Postmortem

 ** Presenting Author*

K14 Phenazepam and Driving Impairment: A Case Report

Paige L. Hinners, BS, 1000 Gladstell Rd, #508, Conroe, TX 77304; Monica Brady Mellon, MS, 8301 New Trails Dr, Ste 125, The Woodlands, TX 77381; and Sarah Kerrigan, PhD, SHSU Regional Crime Lab, 8301 New Trails Dr, Ste 125, The Woodlands, TX 77341*

After attending this presentation, attendees will understand the potential for phenazepam, a lesser known 1,4-benzodiazepine to impair driving performance.

This presentation will impact the forensic science community by providing an increased understanding of phenazepam impairment, specifically the extent to which it may impair some individuals at low dose.

Phenazepam is a 1,4-benzodiazepine that is structurally related to lorazepam and bromazepam. It originated in the Soviet Union in the 1970s and recently emerged as a drug of abuse. It is reported to be one of the most frequently prescribed benzodiazepines in Russia and other Commonwealth of Independent State (CIS) countries. Although it has no legitimate clinical uses in the United States, it has been used therapeutically for its sedative hypnotic, anticonvulsant, muscle relaxant, anxiolytic, and for the treatment of alcohol withdrawal overseas. When used therapeutically, it is available as 0.5mg and 1mg tablets, injectable solutions (0.1% and 0.3%) and transdermal patches. Oral doses of 0.5mg (2 – 3 times daily) may be prescribed, but doses up to 10mg/day are reported.

Several eastern European countries have taken steps to control phenazepam. Here in the United States, it is not controlled at the Federal level, although two states (Arkansas and Louisiana) enacted recent legislation to control the drug. Illicitly, it is available as a powder, tablet, and blotters (similar to LSD). Recreational users report doses of 2mg – 10mg of the drug. There have been relatively few pharmacological or toxicological studies involving phenazepam. In one study involving doses of 3mg – 5mg, peak plasma concentrations of 24 ng/mL – 38ng/mL were observed at approximately 4 h with a half-life of approximately 60 h. When 2mg doses were administered intramuscularly in epileptic patients, the half life was estimated to be 15 h. Adverse effects may include somnolence, dizziness, incoordination, and asthenia.

In a report from Finland, 3.4% of all Driving Under the Influence of Drug (DUID) cases were found to contain phenazepam. In the vast majority of cases (77 of 83 positive cases), other drugs were also detected. Multiple drug use can complicate interpretation, particularly for drugs that are less studied. Performance deficits attributed to phenazepam include unstable gait, confusion, impaired balance, slurred speech, memory loss, ataxia, and pupils that are slow to react to light.

A case is reported of a 24-year-old male apprehended for impaired driving. The subject failed to stop at an intersection and was involved in a two-vehicle crash. The subject had slurred speech and profound psychomotor impairment. His balance was poor; he staggered, and after being placed in a chair, was unable to stand without falling. Blood toxicology was initially negative at another laboratory. The sample was sent to SHSU Regional Crime Lab for additional testing due to the inconsistent results. Comprehensive toxicology testing by Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) revealed the presence of phenazepam at a concentration of 76ng/mL in blood. No other drugs were detected. Phenazepam was quantitated using an Agilent HP 5975 MSD/6890 GC with a HP-5MS capillary column (30m x 0.25mm x 0.25µm). In the absence of deuterated phenazepam, prazepam was used as the internal standard.

The immunoassay cross-reactivity of phenazepam was investigated and found to be >250% using the immunalysis benzodiazepine Enzyme Linked Immuno-Sorbent Assay (ELISA) used in the laboratory. Due to the high cross-reactivity, the sample screened presumptively positive at the 50ng/mL (oxazepam) cutoff. Initial screening at the first laboratory by Enzyme Multiplied Immunoassay Technique (EMIT) was negative, resulting in no further testing. This case report highlights the importance of cross-reactivity in immunoassay and the need to perform more extensive, broad spectrum screening for impaired driving cases, especially when impairment and toxicology results are inconsistent. In this case report, severe impairment was observed in an individual following phenazepam use. The concentration detected was consistent with a single dose of the drug. Phenazepam is a lesser known low-dose benzodiazepine with the potential for significant traffic safety consequences.

Phenazepam, Impairment, Immunoassay

 ** Presenting Author*

K15 Identification and Quantification of Tapentadol and N-Desmethyltapentadol in Human Urine Using Gas Chromatography-Mass Spectrometry

Shannon Cassatt, MS, 310 Sleepy Hollow Rd, Richmond, VA; and Carl E. Wolf, PhD, and Alphonse Poklis, PhD, VCU/MCVH Station, Medical Coll of Virginia, Box 98-165, Richmond, VA 23298*

After attending this presentation, attendees will learn about a Gas Chromatography-Mass Spectrometry (GC/MS) method developed to identify and quantify tapentadol and its main metabolite N-desmethyltapentadol (NDT) in human urine. Attendees will also understand that preliminary investigations demonstrated that the NDT metabolite does not have the same extraction characteristics and chemical derivatization properties as the parent drug. Therefore, special considerations were necessary when developing a method for simultaneous identification and quantification of tapentadol and NDT in human urine.

This presentation will impact the forensic science community by describing to forensic toxicologists and chemists the first GC/MS method developed and validated to identify and quantify tapentadol and its main metabolite NDT in human urine.

Chronic pain is one of the most persistent health care problems in the United States. When physicians fail to properly address pain in patients, it can lead to additional health problems or decrease the patient's quality of life. In many health care settings, opiate and opioid drugs have become the treatment of choice for pain management because of their effective analgesic properties. Tapentadol (Nucynta®) is a relatively new drug that is approved for treatment of both immediate and chronic pain. Tapentadol causes analgesia by acting as an agonist at the brain's mu receptors and as a norepinephrine reuptake inhibitor. Combining these two mechanisms of action makes tapentadol different from "traditional" opiate and opioid drugs, which do not act as norepinephrine reuptake inhibitors. As an analgesic drug, it is likely that incorporation of tapentadol into pain management and pain-monitoring programs will become more widespread, and it will be necessary for toxicology laboratories to be able to identify and quantify the drug and its metabolite in human urine specimens.

The development and validation of the first GC/MS assay developed for the identification and quantification of tapentadol and its major metabolite NDT in human urine samples will be described. Method development studies were initially performed to design an assay that was optimized for the extraction, identification, and quantification of tapentadol and NDT. The optimized procedure involved sample alkalinization with saturated borate buffer (pH 9.5) and extraction into chloroform: isopropanol (9:1) solvent. Samples were evaporated to dryness under a stream of nitrogen and derivatized with 25µl MTBSTFA + 1% TBDMCS: acetonitrile (1:2) at 55°C for >2h.

Quantification of tapentadol and NDT required two internal standards. Deuterated tapentadol-d3 was used for tapentadol quantification and 4-(2-methylamino)propyl)phenol was used for NDT because deuterated NDT was not commercially available. Two internal standards were needed because of the extraction differences between tapentadol and NDT, arising from the metabolite's secondary amine structure. The secondary amine structure of NDT also limited the compound's solubility in MTBSTFA + 1% TBDMCS derivatizing reagent, making it necessary to solubilize the metabolite with acetonitrile to maximize silation derivatization.

Glucuronide conjugates are the primary route of tapentadol and NDT elimination. Hydrolysis studies were performed to liberate the glucuronide conjugates from urine samples. Tapentadol-β-D-glucuronide and NDT were analyzed using the developed GC/MS program and no free tapentadol was detected. Overnight hydrolysis with helix pomatia H-2 was found to be the optimal hydrolysis method, with approximately 50% tapentadol liberated at concentrations of 150ng/ml, and 47% at concentrations of 600ng/ml.

The assay met all laboratory validation criteria with respect to linearity, sensitivity, accuracy, inter-assay precision, intra-assay precision, selectivity, matrix effects, process efficiency, recovery, bench-top stability, and instrument stability. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for tapentadol and NDT were administratively set at 10ng/ml and 50ng/ml, respectively.

Five quality-control samples were run in triplicate over an eight-day validation period with tapentadol and NDT at concentrations of 50, 150, 600, 1500, and 2500ng/ml. The accuracy of the quality-control samples were within ±16% of the target value and the precision %CV values (inter and intra) were <16%. Matrix effect, process efficiency, and recovery were assessed by analyzing six replicates of tapentadol and NDT at concentrations of 150 and 600ng/ml. At both concentrations, tapentadol recovery was 100% and NDT recovery was 83% at 150ng/ml and 96% at 600ng/ml. The validated method allows for the identification and quantification of tapentadol and NDT in human urine.

Tapentadol, N-Desmethyltapentado, GC/MS

 ** Presenting Author*

K16 Deaths Involving the Recreational Use of α-PVP (α-pyrrolidinopentiophenone)

Lauren L. Richards-Waugh, PhD, Kristen M. Bailey, MS, David J. Clay, BA, Myron A. Gebhardt, MS, Christina L. Newsome-Sparks, BS, Hahmada E. Mahmoud, MD, Susan E. Venuti, MD, and James C. Kraner, PhD, OCME, 619 Virginia St, W, Charleston, WV 25302*

After attending this presentation, attendees will gain a better understanding of potentially fatal consequences involving the stimulant hallucinogen α-pyrrolidinopentiophenone (α-PVP). Attendees will also obtain information addressing the measurement of this compound in human autopsy samples.

This presentation will impact the forensic science community by providing useful information regarding the toxicological analysis of cases involving designer stimulants, highlighting the need to consider the potential involvement of such drugs when presented with suggestive incident circumstances.

Until recently, the most common stimulant hallucinogen used illicitly in the United States was MDMA. However, the rapidly escalating availability of a variety of highly potent analogues has resulted in drug-related morbidity and mortality including violent confrontations, motor vehicle accidents, suicides, and fatal drug toxicity. A group of these compounds, collectively referred to as "bath salts," have been sold via the internet and through a variety of retailers including gas stations, convenience stores, and so-called "head shops." Typically, the compound present in these preparations has been 3,4-methylenedioxypyrovalerone (MDPV). Over a three-month period beginning in March 2012, the West Virginia Office of the Chief Medical Examiner has investigated three deaths involving a similar drug, α- α-PVP. The mechanism of action of this drug is thought to involve inhibition of the reuptake of norepinephrine, dopamine, and serotonin. Here is reported case circumstances and toxicological findings in three deaths involving α-PVP in which the decedents exhibited aggression, paranoia, violence, and homicidal behaviors.

Samples obtained at autopsy underwent routine postmortem toxicological testing. This included blood alcohol analysis by Gas Chromatograph/Flame Ionization Detector (GC/FID), drugs of abuse by immunoassay, Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) screening of blood precipitates, and Gas Chromatograph/Mass Spectrometry (GC/MS) screening of alkaline and acidic/neutral blood extracts. GC/MS analysis of alkaline extraction of urine utilizing Toxi tube A was helpful in identifying the presence of α-PVP. Confirmation and quantitation of α-PVP was performed by GC/MS analysis of an alkaline liquid-liquid extract (without derivatization).

The decedents were adult males aged 31, 35, and 51 years. The oldest male was found deceased on his bathroom floor. Empty packages of bath salts were discovered at the scene with yellowish-tan powder noted within the nostrils. The most significant toxicological finding was α-PVP at a concentration of 0.10mg/L in the blood. THC and carboxy-THC were also present at 2.6 and 25ng/mL, respectively. The second fatality involved witnessed seizure activity preceding the demise. A history of bath salt and prescription drug abuse was reported. Toxicology results included α-PVP at a concentration of 0.52mg/L in the blood in addition to sertraline (0.16mg/L), oxycodone (0.02mg/L), and 7-aminoclonazepam (<0.01mg/L). The youngest male died from firearm injuries during an armed confrontation with law enforcement involving aggressive and paranoid behavior, as well as suicidal threats. Vials believed to contain bath salts were found in the decedent's pockets. Drugs confirmed in the blood included α-PVP and pentedrone at concentrations of 0.29 and 0.48mg/L, respectively. In all three cases, α-PVP was deemed to be the primary cause of death or a significant contributory factor.

Current routine postmortem toxicological analysis may not detect many of the designer stimulant compounds that present an increasing challenge in forensic pathology and toxicology. Often, cases in which the history documents bizarre, aggressive, hallucinogenic, or paranoid behaviors, and/or symptoms consistent with overstimulation of the sympathetic nervous system are positive for compounds such as α-PVP or other cathinone derivatives upon targeted analysis. Similar to other sympathomimetic drugs, establishing toxic and lethal concentrations for α-PVP will likely be difficult. These drugs often demonstrate significant overlap between concentrations tolerated by individuals and those reported in drug-related fatalities. Investigative history, autopsy findings, and toxicology results must be fully assessed to most accurately determine the cause and manner of death in cases involving designer stimulants such as α-PVP.

α-PVP, Bath Salts, Postmortem

 ** Presenting Author*

K17 Two Cases of Suicide in Nurses by Atracurium: Revealed by LC/ESI/MS

Carmela Fiore, MD, Viale degli Aviatori, 1, Dept of Forensic Pathology, Univ of Foggia, Foggia, Italy 71100, ITALY; Francesca Giannelli, Ospedale Colonnello D'Avanzo, Foggia, ITALY; Palmira Fortarezza, Ospedale Tatarella, Cerignola, ITALY; Eugenia Di Stefano, MD, Ospedale Colonnello D'Avanzo, Foggia, ITALY; and Fabio Centini, BS, Policlinico Le Scotte, Siena, ITALY*

The goal of this presentation is to show two suicidal cases of atracurium in nurses, revealed by Liquid Chromatography/Electrospray Ionization/Mass Spectrometry (LC/ESI/MS). The analytical method and the postmortem toxicological concentrations of atracurium and laudanosine revealed in both fluids and tissues are discussed.

This presentation will impact the forensic science community by showing the importance of an analytical method developed for simultaneously quantifying postmortem of atracurium and its metabolite laudanosine in two suicidal cases.

Atracurium is a non-depolarizing skeletal muscle relaxant. It is a derivative of curare, a plant extract prepared from many different plants of the Amazon forest, used by the natives of the area as a poison arrow for hunting and war. It is used to facilitate endotracheal intubations and to relax skeletal muscles during surgery or mechanical ventilation. It is available as a 1% solution of the besylate salt for intravenous administration. It can be fatal in any concentration due to respiratory failure, so controlled ventilation is necessary. Following an intravenous dose, the muscles begin to relax within about two minutes and the effect lasts for 15 min – 35 min, depending on the dose. The drug is excreted in urine and bile, and its elimination half-life is around 20 min.

This presentation concerns two lethal cases of polydrug intoxication, both positive for the atracurium:

- The first case (named "A") involved a nurse of the Emergency Unit found dead in his home. Near his body, a syringe containing few cc's of colorless liquid and an empty blister pack of tablets of midazolam were found.
- The second case (named "B") involved to a nurse found unresponsive in the hospital where he was employed. Near his body, a syringe containing 11cc's of colorless liquid and an empty bottle showing the words "sodium pentothal" were found.

A comprehensive toxicological screening was performed on postmortem cardiac blood, urine, bile, and tissue homogenates (liver, heart, and kidney) using a combination of immunoassay and chromatographic techniques.

In detail, in both cases, lethal concentrations of midazolam were confirmed in biological fluids and tissues of the body A, while the presence of thiopental was revealed in biological fluids and tissues of the body B.

Since atracurium degradation occurs rapidly *in vitro* by the same hydrolysis mechanism observed *in vivo*, and it is accelerated by an alkaline pH and high temperatures, and given its simultaneously precharged yet lipophilic nature, detecting low atracurium levels in human postmortem samples is a challenge.

A method was developed for simultaneously quantifying low levels of atracurium and its less polar metabolite laudanosine in postmortem blood, bile, urine, and tissues by LC/MS in an ion trap mass spectrometer under positive ion ESI conditions. Analytes were isolated from blood and tissues by solid-phase extraction using Bond-Elut Certify columns. The method proved selective and sensitive, and was validated in postmortem blood, bile, urine, heart, kidney, and liver in the range of 1 – 2000ng/mL (blood) and 5 – 5000ng/g (tissues). The proposed method was fully validated with respect to previously published LC/MS methods.

Lethal concentrations of atracurium and laudanosine were confirmed in all the biological fluids and tissues of both bodies. The presence of atracurium was also confirmed by toxicological examination of the colorless liquid found in syringes.

Based on the autopsy findings, case history, and toxicology results, the forensic pathologists ruled that the cause of death in both cases was an overdose of atracurium in combination with midazolam for body A and thiopental for body B; the manner of death was suicide.

Atracurium and Laudanosine, Liquid Chromatography, Toxicological Finding

 ** Presenting Author*

K18 Direct Analysis in Real Time (DART®) Analysis With a Modified GC/MS System for Rapid Drug Screening

Brian Musselman, PhD, 999 Broadway, Saugus, MA 01906*

After attending this presentation, attendees will learn about complete rapid screening for drugs of abuse using Direct Analysis In Real Time (DART®) screening capability with their Gas Chromatograph/Mass Spectrometry (GC/MS). This is important since traditionally the DART® technology has required a more complex Liquid Chromatography/Mass Spectrometry (LC/MS) for operation.

This presentation will impact the forensic science community by enabling more rapid screening of samples with existing GC/MS systems that are prevalent in the community. This technology can speed identification of drugs of abuse, reduce turn-around-time, and reduce sample backlogs.

DART® is an ambient ionization method that provides rapid determination of sample composition with little sample preparation. Samples are directly sampled and ionized merely by placing the material in the flow of heated ionizing gas. Solids or liquids are readily analyzed, often without any manipulation or purification of the sample. The ionization usually occurs by the excited helium atoms reacting with ambient water to form protonated water clusters. These water clusters attach a proton onto the molecule of interest, producing a spectrum that is very simple and often composed of one peak per compound. This leads to the facile interpretation of the spectra and the ability to analyze mixtures without the complexity of many fragment ions.

Several major forensics laboratories including FBI, Secret Service, the FDA Forensic Chemistry Center, and the Virginia Department of Forensic Science, have utilized DART® for rapid detection and characterization of unknowns. Published papers show analysis of gamma-hydroxybutyric acid, synthetic cannabinoids, analysis of sexual assault evidence, alprazolam tablets, methamphetamine, bank security device and pepper spray components, explosives trace detection, ricin activity assay, iodine and red phosphorus, and chemical warfare agents.[1-10]

However, this time-saving ambient ionization technology has not gained a wider audience in the forensic community for several reasons. A major reason is the fact that the DART® source requires a mass spectrometer equipped with an Atmospheric Pressure Inlet (API) typically supplied with an LC/MS system. Since this technology is limited to more specialized laboratories, the analysts cannot readily access the current DART® technology. In this current effort, an API has been integrated into an Agilent Mass Selective Detector (MSD), which is widely used in state and federal laboratories for trace forensic analysis. Secondly, the sampling process has been left to the analyst, allowing for flexibility, but also encumbering the analysts with additional method development. The sample preparation process has been simplified with a new device composed of a card-containing metal screen that holds the liquid or solid sample. The sample is placed on the screen, the card is inserted into the source, and the spectrum acquired in less than 10 seconds.

Facilitating DART® analysis with the low cost Agilent mass analyzer should enable more laboratories to add this capability, speeding analysis and reducing backlogs. This presentation will demonstrate the application of this modified DART®-MSD for determination of the presence of drugs in urine with a simple solid phase extraction for sample preparation. This reduces analysis time of 30 – 60 min to less than one minute. Additionally, the direct analysis of solid dosage forms of drugs of abuse will be illustrated, showing how DART® can identify these materials in seconds.

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Drugs of Abuse, DART®, Mass Spectrometry

 ** Presenting Author*

K19 Survey of Practices in Toxicological Investigation of Drug-Impaired Driving

Kayla Lowrie, MS, 520 Stevens Dr, Apt 208, King of Prussia, PA 19406; Jennifer L. Turri, BS, W 3901 Johnson Rd, Vulcan, MI 49892; Jillian K. Yeakel, MS, 105 Revere Dr, Harleysville, PA 19438; and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to compare their laboratory's practices with peer laboratories and evaluate typical cutoffs used for drug screening and confirmation. This presentation will describe data from a survey carried out to evaluate the practices of forensic toxicology laboratories performing analysis in the investigation of Driving Under the Influence of Drugs (DUID) cases. The survey was sponsored by the National Safety Council's Committee on Alcohol and Other Drugs (NSC CAOD).

This presentation will impact the forensic science community by providing data to support updating of general recommendations for laboratory testing in DUID investigations in order to improve consistency and standards of screening and confirmation.

The purpose of this survey was to evaluate scope and sensitivity of testing, compliance with the current recommendations for DUID testing, and changes in patterns of drug use by drivers in DUID investigations that might warrant updating of previous recommendations. This research aimed to assist in critically reviewing and updating the current guidelines and recommendations for the toxicology community.

An online web-survey instrument was used. The survey questions focused on scope and sensitivity for drug screening and confirmation, analytical methods, and ability to meet previously published recommendations.[1] The final revised survey was sent to confirmed participants via the online survey. Follow-up emails and phone calls were used to obtain additional information or clarify responses. In spite of these efforts, some participants did not respond to all questions; therefore, the data represents 96 surveys completed to the point where they were deemed sufficiently complete for inclusion in the data analysis.

It was indicated that 80% of responding labs test blood samples and 68% reported testing urine samples in DUID casework. Few labs reported testing oral fluid, and not consistently. Screening methods for blood testing were mostly Enzyme-Linked Immuno-Sorbent Assay (ELISA) (34%), Gas Chromatograph/Mass Spectrometry (GC/MS) (28%), Liquid Chromatography/Mass Spectrometry (LC/MS) (17%), and Enzyme Multiplied Immunoassay Technique (EMIT) (13%). No labs reported using Liquid Chromatography Time-Of-Flight (LCTOF) screening for blood. For urine, 29% reported GC/MS screening, ELISA (27%), EMIT (23%), and LC/MS (14%). For confirmatory testing, 52% of labs reported using GC/MS, while 36% used LC/MS. Labs were asked about reporting unconfirmed results, and 33% indicated they would report those under some circumstances, including insufficient sample, lack of a confirmatory procedure (with a recommendation to have testing sent out), and emphasized the inclusion of disclaimer about the presumptive nature of the result.

Respondents were asked whether their laboratories practices were consistent with the 2007 recommendations. Responses varied by drug and matrix. For screening purposes, the majority of labs reported meeting or exceeding the guideline recommendations for drugs of abuse, including carboxyTHC, benzoylecgonine, benzodiazepines, MDA, barbiturates, methadone, opiates, and PCP. The majority did not meet the recommendations for amphetamines. Drugs for which the majority of laboratories did not meet the recommendations for confirmatory testing were mostly therapeutic drugs including trazodone, nortriptyline, carisoprodol, zolpidem, topiramate, and methadone.

Participants were asked to indicate which additional drugs should be included in the recommendations for routine screening and confirmation. At least 75% of the 68 participants who responded to this question indicated that mephedrone, zopiclone, and buprenorphine should be included in future recommendations for blood sample screening. Additionally, at least 50% of the participants indicated that methylone, MDPV, JWH-073, JWH-250, JWH-081, JWH-122, JWH-210, JWH-019, JWH-200, AM-2201, benzylpiperazine, trifluromethylphenylpiperazine, dimethyltryptamine, modafinil, quetiapine, and zaleplon should be included in the future recommendations for blood sample screening.

Based on this input, the NSC CAOD is updating the guidelines for distribution early in 2013.

Reference:

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DUID, Cutoffs, Guidelines

 ** Presenting Author*

K20 Simultaneous Quantification of Amphetamines, Ketamine, and Opiates in Urine Using SPE and LC/MS/MS

ChuAn Yang, MA, Institute of Forensic Medicine, 123, Min-An St, Zhonghe Dist, New Taipei City, Taiwan, TAIWAN, ROC; Hsiu-Chuan Liu, MS, Taipei, TAIWAN, ROC; Ray H. Liu, PhD, 3737 Rivera St, San Francisco, CA 94116; and Dong-Liang Lin, PhD, Institute of Forensic Medicine, 123, Min-An St, Zhonghe Dist, New Taipei City, Taiwan 23548, TAIWAN, ROC*

After attending this presentation, attendees will learn of a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) technique for analyzing amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine, and norketamine in urine.

This presentation will impact the forensic science community by developing a simple, accurate, and fast analytical method of LC/MS/MS capable of quantifying ten analytes in urine that are abused drugs.

Heroin, methamphetamine, and ketamine have historically been the most commonly abused drugs in Taiwan and are routinely monitored in the laboratory by Gas Chromatography/Mass Spectrometry (GC/MS) methods. The purpose of this study was to evaluate whether the LC/MS/MS-based approach can be more effectively applied to the simultaneous quantitation of amphetamine (AM), methamphetamine (MA), MDA, MDMA, morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM), 6-acetylcodeine (6-AC), ketamine (K), and norketamine (NK) in postmortem urine specimens.

Samples (1mL) were extracted via solid-phase extraction, evaporated, and reconstituted in the mobile phase for injection onto the LC/MS/MS system. Deuterated analogues of the analytes of interest were used as internal standards. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100mm ´ 2.1mm i.d., 1.8-mm particle) analytical column at 50°C. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was held for 1.5 min, then decreased to 0% A in 8.5 min and held for 2 min, then increased to 90% A in 1 min and held for 2 min. MS analysis was performed by an electrospray ionization in positive-ion Multiple Reaction Monitoring mode (MRM) with optimized collision energy for the precursor ion selected, monitoring two transitions for each analyte.

Validation was performed by extracting drug-free urine fortified with 50 – 1000ng/mL of the 10 analytes, yielding the following results: (1) average extraction recovery (n=5) was >80%, except for MDMA (70%) and MOR (74%); (2) inter-day and intra-day precision ranges (%CV) were 1.59 – 9.13% and 0.57 – 3.89%; (3) calibration linearity (r^2), detection limit, and quantitation limit were >0.997, 1ng/mL and 5ng/mL for all analytes, respectively; and, (4) matrix effects: ion suppression was lower than 20% for all analytes; it was compensated by using deuterated internal standard. Compared with traditional GC/MS methods, the conclusion arose that this relatively simple protocol can be used for routine and reliable identification and quantitation of AM, MA, MDA, MDMA, MOR, COD, 6-AM, 6-AC, K, and NK in urine. This method was successfully applied to the analysis of postmortem and antemortem specimens from forensic cases.

Drugs of Abuse, Urine, LC/MS/MS

 ** Presenting Author*

K21 Fatal Cases of Aconitum Alkaloids Poisoning in Korea

Sujin Jeong, MS, 139 Jiyang-ro Yangcheon-gu, Seoul, Korea 158-707, KOREA; Seungkyung Baeck, PhD*, 139 Jiyang-ro Yangcheon-gu, Seoul, Korea 158-707, KOREA; Sangki Lee, PhD*, 139 Jiyang-ro Yangchun-gu, Seoul, Korea , KOREA; Hee-Sun Chung, PhD*, 1402-703 Mokdong Apt, Shunjung-dong, Yangchon-ku, Seoul, Seoul 158-774, KOREA*

After attending this presentation, attendees will gain knowledge regarding concentration levels in fatal cases of aconite poisoning and detection methods.

This presentation will impact the forensic science community by showing how this method was successfully applied to detect aconitines in various specimens of related aconitum alkaloids poisoning cases.

Aconitum alkaloids have been occasionally used in Korean herbal medicine because of pharmacological effects such as analgesic, anti-epileptic, and anti-inflammatory, but they can lead to sudden death by their cardiotoxins and neurotoxins. In traditional medicine, aconite roots are used only after processing to reduce the toxic alkaloid content. Soaking and boiling during processing will hydrolyze aconite alkaloids into less toxic and non-toxic derivatives; however, the use of a larger-than-recommended dose and inadequate processing increases the risk of poisoning. Every year, several causes of death were contributed to aconite toxicity. The high levels of toxicity of aconite are considered to be derived from aconitine, mesaconitine, and hypaconitine. The lethal dose of aconitine in human adults is estimated to be only 1mg – 4mg.

There have been reported cases of homicide, suicide, and accidental ingestion. Severe aconite poisoning can occur after accidental ingestion of wild plants or consumption of herbal decoction-made aconite roots. Some fatal cases were caused by unrefined herbal medicine prepared from aconite. Aconitum alkaloids have been indentified in various samples such as traditional prescribed herbal medicine, aconite infusion water, aconite liquor, wild greens mixed aconite, and their biological specimens from five cases related to aconite poisoning this year.

A rapid, specific, and sensitive Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed for simultaneous identification of aconitine, mesaconitine, and hypaconitine. The determination of aconitum alkaloids in specimens was performed by LC/MS/MS after liquid-liquid extraction using trimipramine-d3 as an internal standard. Samples of each, 1mL or 1g, were extracted with 5mL of ethyl acetate in alkali of NH_4OH. The organic layer was dried with a stream of nitrogen at 45°C. The residues were reconstituted with methanol and injected into LC/MS/MS. The separation was applied on Agilent XDB C18 column (1.8 micron, 4.6×50 mm). The injection volume was 5µL and the retention time was less than 8 minutes. A gradient elution of acetonitrile and water of 0.1M ammonium formate and 0.1% formic acid were used as mobile phase. Flow rate was 0.4mL/min. LC/MS/MS system (ABSciex, ABI 3200QTrap) coupled with an Electrospray Ionization (ESI) source was performed in multiple reaction monitoring(MRM) mode. The transitions of the Aconitum alkaloids executed as follows: m/z 646.3→586.0 for aconitine, m/z 632.3→572.4 for mesaconitine, m/z 616.3→556.3 for hypaconitine, and m/z 298.3→103.0 for trimipramine-d3 as internal standard. This method was successfully applied to detect aconitines in specimens. The validation results of selectivity, matrix effect, recovery, linearity, intra- and inter-assay precision, and accuracy were satisfactory.

It is well known that aconite poisoning can cause various symptoms, including arrhythmia and death, but specific autopsy findings are not configurative. There is a potential risk to overlook the death by aconite ingestion without advance information. When given more information about the scene, this method is useful to investigate aconite poisoning. At the same time, the public should be warned of the danger of eating wild plants and be educated on the potential hazards from self treatment with aconite root. In addition, it is necessary to have institutional restrictions on aconite medicine.

Aconitine, Fatality, LC/MS/MS

 ** Presenting Author*

K22 Flight Activity and Drug Use: Legislation and Toxicological Statistics From 2006 – 2012 at the Rome Medical Legal Institute of the Italian Air Force

Paola Frati, PhD, Dept of Anat Histol Forensic and Orthop Sciences, Univ of Roma, La Sapienza, Viale Regina Elena 336, Roma, 00161, ITALY; Angelo Landolfi, Via Atene 59, Ciampino, Roma 00043, ITALY; Paola Mancarella, and Arianna Giovannetti, Viale Regina Elena 336, Rome, ITALY; Francesco P. Busardo, MD, Via Del Vespro, 129, Palermo, ITALY; Giuseppe Ciniglio Appiani, MD, and Raffaele Cresta, MD, Via Piero Gobetti 2/A, Roma, ITALY; and Domenico Abbenante, MD, Via Largo Claudio Monteverdi, Cerveteri, ITALY*

After attending this presentation, attendees will understand the medical assessment of commercial pilots and cabin crews, which has two main purposes: (1) to assess their functional ability; and, (2) to ascertain whether they are physically able to safely exercise the privileges of their licenses and to verify the risk of incapacitation during the period of validity of the medical certificate.

This presentation will impact the forensic science community by demonstrating the importance of continuous surveillance of commercial pilots and cabin crews. As this study confirms, the percentage of drug users in this category of workers is very low.

The goal of this presentation is to describe the experience of the Italian Air Force Medicolegal Institute of Rome and the Forensic Laboratory of the Catholic University regarding the medical assessment of commercial pilots and cabin crews.

Materials and Methods: The total number of Class 1 and 2 medical examinations undertaken during a six-year period from January 2006 to the first semester of 2012 was taken from the Italian Air Force Medicolegal Institute of Rome medical records database.

The normative references in the relevant period regarding personal fitness to fly are: Italian Presidential Decree n. 566 November 18, 1988; JAA JAR-FCL 3 Flight Crew Licensing (medical) Amendment 5, December 1, 2006.

Urinary screening for the qualitative detection of drugs was carried out using the immunochemical technique Kinetic Interaction of Microparticles in a Solution (KIMS). The following substances were tested for: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates. All samples were processed to guarantee chain of custody, obliging operators to document the different stages of the sample. Samples were separated into two aliquots (sample and counter-sample) and closed in front of the patient with a tamper-proof seal signed by the healthcare operator and the patient. The counter sample of urine that tested positive in a preliminary analysis was kept in the freezer at -20°C for 60 days, to be used in case any medical-legal disputes arose.

Results: The results of preliminary analysis of the urinary specimens were then examined and elaborated. Data review allowed the evaluation of the sample distribution by gender, age, drug substance type, with subsequent confirmation by Gas Chromatography/Mass Spectrometry (GC/MS). Within the positive samples, analyzed using the KIMS, the gender distribution is almost equal (five male subjects, compared to four female subjects) with an age range of 19 – 50 years.

In the relevant period, of 7,530 subjects tested for drug use (only extraordinary medical examination), nine were positive to KIMS screening. Among the positive subjects, none were polydrug users. Distribution of positive results for drugs indicated a clear prevalence of cannabinoids (eight subjects, or 89%). Only one positive case was detected for cocaine (11%) and no samples were positive for barbiturates, benzodiazepines, amphetamines, or opiates.

The cases that were positive after urinary screening, and their samples, were then subjected to confirmation by GC/MS. Of the nine positive cases, five cases (equal to 56% of all positive) haven't been confirmed. In the four confirmed cases, one was detected for cocaine and three for cannabinoids.

Discussion and Conclusion: Thanks to the continuous surveillance of commercial pilots and cabin crews, the study shows that the percentage of drug users is very low; therefore, this result indicates that it is appropriate to continue this strict type of monitoring. This phenomenon should not be underestimated since it can influence the ability of individuals who are responsible for the safety of others.

Substances of Abuse, Italian Air Force, Toxicological Investigation

 ** Presenting Author*

K23 Blood Transfusions and Their Influence on the Evaluation of Postmortem Alcohol Levels in Biological Fluids in Road Traffic Accidents: Case Report And Review of Literature

Francesca Pepe, MD, Viale Europa, Località Germaneto, Catanzaro, Italy 88100, ITALY; Isabella Aquila, MD*, Viale Europa, località Germaneto, Policlinico Universitario, S. Venuta- Medicina Legale, Catanzaro, 88100, ITALY; Ciro Di Nunzio, MFS, PhD, Magna Graecia Univ, Viale Europa, Germaneto, Legal Medicine, Catanzaro, 88100, ITALY; Silvia Boca, MD, Viale Europa 88100 Catanzaro, Catanzaro, 88100, ITALY; Maria Chiarelli, MD, and Gratteri, MD, Viale Europa, Germaneto, Catanzaro, 88100, ITALY; and Pietrantonio Ricci, PhD, Viale Europa, Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will understand the relationship between blood transfusions and postmortem alcohol levels in biological fluids.

This presentation will impact the forensic science community by discussing drunk driving and its social consequences.

Introduction: Traffic accidents are an important cause of death, particularly among young people. To drive under the influence of alcohol and/or drugs is the cause of many of these accidents. In particular, the alcohol concentrations are measured in a corpse through the toxicological postmortem analysis on the body fluids. The blood represent the biological liquid that allows verification of drunk driving at the time of the accident. In particular, the concentration of alcohol in the blood is subject to variations due to different etiological factors. Blood alcohol levels in the corpse are altered by the putrefactive phenomena. Also, blood transfusions may influence the concentration of blood alcohol levels. This medical practice is performed to restore hemodynamic parameters in patients with critical conditions, usually after a traffic accident. The exact determination of blood alcohol levels is very difficult to determine during the autopsy examination of the corpse which has been exposed to the blood transfusion procedure.

Objective: The goal of this study is to examine the influence of blood transfusion on blood alcohol levels in cases of deaths from traffic accidents. In particular, the focus is on the reliability of postmortem toxicology data in subjects during the last stages of life who have been transfused to be resuscitated.

Case report: The study investigates a 36-year-old subject, hospitalized in the emergency room for traumatic shock after a traffic accident. He underwent surgery for left nephrectomy because of renal laceration, but died of these injuries: left temporo-parietal hemorrhage with subarachnoidal hemorrhage; bilateral hemothorax; fracture of the right clavicle; multiple rib fractures with bilateral pulmonary parenchymal contused injuries; fractures of the fifth dorsal vertebra without spinal cord injury; and, liver and intestinal lesions.

Results of Toxicological Investigations: Toxicological analysis performed on body fluids showed high levels of ethanol in bile, vitreous humor, and blood, as well as high levels of methadone. The values of ethyl alcohol in the blood were of 2.29g/l. Because of blood transfusion, the concentration of alcohol in vitreous humor had to be estimated. In effect, the vitreous humor levels should be in equilibrium with blood levels. The vitreous humor levels were the lesser affected of the two because the blood had been diluted by transfusion. Additionally, because the value of ethyl alcohol in the vitreous humor reaches a chemical equilibrium with a ratio of about 1:1 including the lymph and circulatory fluids, the vitreous humor levels were less affected. Many calculations were carried out to evaluate the value of ethyl alcohol in the blood at the time of the first transfusion. This result is shown through the application of appropriate correction factors that have considered the amount of blood transfused (1400ml), the weight of the subject, the metabolism of alcohol, the metabolic capacity medium, and the time elapsed from ingestion to accident. These calculations have determined the value of hypothetical blood alcohol at the time of the car crash before transfusion (1.28g/l).

Conclusions: In this case, it was possible to determine the concentration of blood alcohol levels over and above the cut-off. It has been concluded that the person was driving under the influence of alcohol. This investigation is essential for judicial purposes, in particular when it comes to an accident involving people who are the driver's responsibility. The study allows evaluation of a theme that has great social impact—it is very important to evaluate the conduct of the driver at the time of the incident.

Alcohol, Blood Transfusion, Toxicological Investigation

 ** Presenting Author*

K24 Preclinical Investigation of CP47,497: A Widely Abused Synthetic Cannabinoid

Kimberly Samano, MFS, 6731 Forest Hill Ave, Richmond, VA 23225-1801; Aron H. Lichtman, PhD, 1220 E Broad St, Richmond, VA 23298; and Alphonse Poklis, PhD, VCU/MCVH Station, Medical Coll of Virginia, Box 98-165, Richmond, VA 23298*

The goal of this presentation is to educate attendees about CP47,497 (2-[(1R,3S)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol), a synthetic cannabinoid recently banned by the DEA. The pharmacology of this compound was first described in the scientific literature in 1982, but prior to its ban was being sold for consumption primarily through internet sources or head shops and was associated with a large spike in emergency department visits. CP47,497 activates the cannabinoid receptor 1 (CB_1R) and dose-dependently elicits cannabimimetic effects that are more potent than effects produced from Δ^9- tetrahydrocannabinol (THC). Importantly, these studies provide novel evidence using a whole animal model that the CB_1R antagonist rimonabant reverses the potent cannabimimetic effects of CP47,497

This presentation will impact the forensic science community by providing a direct comparison of synthetic cannabinoid and THC behavioral data in whole animal studies.

CP47,497 and other synthetic cannabinoid compounds were originally synthesized as tools to investigate the mechanism by which marijuana affects the brain as well as for the development of potential therapeutic agents to treat pain and other disorders. However, studies addressing the behavioral consequences of synthetic cannabinoids are scant. Synthetic cannabinoids pose an enhanced risk for abuse, toxicity, and addiction due their increased potency and efficacy over THC. The goal of the present study was to determine whether the pharmacological effects of CP47,497 are achieved in a dose-dependent and time-dependent manner. Since CP47,497 binds to CB_1 receptors and elicits THC-like effects, it was investigated whether rimonabant would attenuate its pharmacological actions *in vivo*.

All mice received intraperitoneal injections of CP47,497, THC, or vehicle. To test for cannabimimetic subjective effects, a tetrad model was utilized that consisted of four outcome measures: catalepsy; antinociception (tail flick latency); hypothermia; and, locomotor activity. Although many pharmacological agents can produce one or a subset of these effects, drugs that activate CB_1 receptors produce measurable effects in all four parameters of the tetrad. Immediately following behavioral testing, mice were humanely euthanized and blood and tissue were harvested for CP47,497 quantification. Samples are currently being analyzed on an Applied Biosystems Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) interface utilizing electrospray ionization and selective ion monitoring, using an acetonitrile liquid-liquid extraction procedure that the laboratory has previously developed validated methods for quantification of THC and other cannabinoids in blood and tissue.

In the cumulative dose-response experiment, mice were treated with THC (3, 10, 30, 100, and 200mg/kg), CP47, 497 (0.3, 1, 3, 10 and 30mg/kg), and vehicle control. Potency ratios for comparison of CP47,497 to THC were calculated including 95% confidence limits for each: catalepsy 7.49 (5.72 – 9.76), antinociception 9.11 (3.76 – 21.98), and hypothermia 7.68 (4.55 – 12.83), which clearly demonstrate CP47,497's enhanced potency and efficacy. In the final component of the tetrad, 30mg/kg CP47,497 produced a statistically significant increase in locomotor depressing effects versus control. Based on the data obtained from the dose-response study, 30mg/kg CP47,497 and 100mg/kg THC were used in subsequent antagonism studies. Both 30mg/kg CP47,497 and 100mg/kg THC produced statistically significant increases in catalepsy, hypothermia, antinociception, and a decrease in locomotor activity versus control. CP47,497 and THC-induced catalepsy and hypothermia were reversed by pretreatment with 3mg/kg rimonabant. Although 3mg/kg rimonabant antagonized the antinociceptive effects of 100mg/kg THC, 10mg/kg rimonabant was required to block the antinociceptive and locomotor depressing effects of 30mg/kg CP47,497.

This study's results provide the first *in vivo* evidence that the cannabimimetic effects of CP47,497 are CB_1 mediated as blockade of these effects is achieved with the CB_1 antagonist, rimonabant. Given that CP47,497 elicits dose-dependent cannabimimetic effects that are markedly (7 – 9 times) more potent than THC-containing substances, these data are consistent with the large number of abusers of this compound presenting with severe cannabis-related adverse effects that require emergency department interventions.

CP47,497, Spice, THC

 ** Presenting Author*

K25 DART® AccuTOF™: A New Drug Screening Protocol for Biological Specimens

Rachel C. Beck, BS, 2026 Valleyday Rd, Hoover, AL 35244; Maggie W. Morgan, HSD, 806 Hillyer High Rd, Anniston, AL 36207; and Tracy Segrest, PhD, and Erin M. Shonsey, PhD, 2026 Valleydale Rd, Hoover, Alabama 35244*

After attending this presentation, attendees will learn how the DART® AccuTOF's™ technology can be utilized as a comprehensive screening technique of whole blood.

This presentation will impact the forensic science community by expanding the scope of analysis of whole blood drug screening to include targets not covered by traditional immunoassay techniques.

The field of forensic toxicology is never stagnant; preferences for a specific drug and/or drug combination fluctuate within the population. Meanwhile, scientists in the field are charged with providing timely, comprehensive, and accurate results while enduring dwindling personnel and financial resources, which often necessitates a limited scope covering only the staples. In order to comply with the duty of identifying both conventional and emerging drugs that are problematic in society, new methodology must be adopted in the screening of biological samples.

Current drug screening practices utilize both traditional immunoassay methodology and Gas Chromatography/Mass Spectrometry (GC/MS) technology. Immunoassays have been successful for the analyses of conventional drugs; however, immunoassays are costly, making scope expansion outside common drugs of abuse monetarily impractical. Additionally, immunoassays are limited for their ability to adapt quickly in the analysis of new and/or additional compounds due to kit production and/or validation. The GC/MS has less scope limitations when compared to the immunoassay but is a great deal more costly with respects to time. GC/MS technology requires tedious sample preparation prior to data collection and consumes multiple days of a scientist's time to complete the extraction, collection, and analysis of the data. Moreover, when used for drug screening purposes, any positive findings must then be confirmed by repetition. The Direct Analysis in Real Time (DART®) ionization source coupled to an AccuTOF™ mass spectrometer offers a solution to the restricted budget, available personnel, and drug screening limitations currently faced. Furthermore, DART® AccuTOF™ technology offers a second methodology for the screening of targets that have been traditionally identified and confirmed by repetitive GC/MS analyses. The robust, open air DART® ionization allows for comprehensive analysis by producing protonated molecular ions for all mode specific (positive mode) ionizable components of the specimen sampled via surface ionization, while the AccuTOF™ mass spectrometer allows for continuous data collection.[1,2] This hybrid instrumentation allows for the putative identification of both parent and metabolite compounds alike via a molecular formula database search with a total instrument analysis time of a couple of minutes per sample.

The application of the DART® AccuTOF™ technologies in the field of toxicology for the screening of whole blood, an exceedingly complex matrix, has realized the necessity for sample preparation prior to analysis.[3] To combat the complexity of the whole blood matrix, Disposal Pipette Extraction (DPX™) tips utilizing a cationic sorbent, featuring sulfonic acid groups, were employed for analysis of basic drugs spiked into porcine whole blood.[4] The amount of blood needed for the analysis was based upon the Limit Of Detection (LOD) study performed with neat standards. Porcine blood was screened for 35 different targets spanning a multitude of drug classes. Detected target coverage included basic and amphoteric compounds in the following classes: cathinones; sympathomimetic amines; select opiates; select benzodiazepines; dextromethorphan; carbamazepine; carisoprodol; select barbiturates; zolpidem; cocaine metabolite; citalopram; tapentadol; and, select tricyclic antidepressants. These detected targets were identified at therapeutic levels ranging from 10ng/mL to 400ng/mL.

Based on the experimental data collected, comprehensive screening can be accomplished with DART® AccuTOF™ technology. This study expanded the current drug screening scope of whole blood beyond the classical impairing drugs and even identified emerging select cathinones (bath salts). In conclusion, DART® AccuTOF™ technology has provided a promising solution to the current drug screening limitations encountered by forensic toxicologists.

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Whole Blood, DART® AccuTOF™, DPX tips

 ** Presenting Author*

K26 More Bang for Your Buck—An Alternative Approach to Blood and Tissue Screening That Saves Time and Money

Mary E. Zaney, BS, 1851 NW 10th Ave, Miami, FL 33136; Diane Boland, PhD, Miami Dade County MEO, Toxicology Laboratory, Number One Bob Hope Rd, Miami, FL 33136; George W. Hime, MS, Miami Dade County MEO, 1851 NW 10th Ave, Miami, FL 33136; and Joseph H. Kahl, BS, 1044 Abell Cir, Oviedo, FL 32765*

After attending this presentation, attendees will have seen an alternative automated solid phase extraction technique to blood and tissue screening as compared to Liquid-Liquid Extraction (LLE) of basic drugs. Better recovery of designer drugs such as "bath salts" will also be shown.

This presentation will impact the forensic science community by demonstrating how more information can be obtained from the same sample volume, while saving time and money.

The Toxicology Laboratory at the Miami-Dade Medical Examiner Department recently changed the blood and tissue screening methodology from a multi-step LLE of basic drugs to a dual-Elution Solid Phase Extraction (SPE) of acidic/neutral and basic drugs. This was done to achieve a more cost-effective comprehensive blood drug screen, which utilizes smaller solvent volume and reduces sample preparation time.

The objective is to present data comparing the previously utilized LLE procedure to the newly implemented automated SPE method. Examples will include spiked controls, proficiency samples, and postmortem cases.

The LLE procedure (described by Forrester et. al in JAT) applies strictly to the extraction of basic drugs from 1mL of sample. The extract is then analyzed by dual column Gas Chromatography with Thermionic Sensitive Detection (GC/TSD).

The SPE method is a modified version of United Chemical Technologies Procedure Code: DRB200DAUZ120392 using UCT Clean Screen® cartridges and an automated Zymark Rapid Trace® system. The procedure uses 1mL sample volume yet yields two distinct fractions. The acidic/neutral extract is submitted for analysis by dual column Gas Chromatograph-Flame Ionization Detector (GC-FID), and the basic extract is analyzed by GC/TSD. GC-Ion Trap/MS is performed to confirm any positive findings.

The SPE method detected all 113 spiked control drugs and showed improved recovery for certain drugs, particularly the sympathomimetic amines and benzodiazepines. Co-elution of doxylamine and etomidate with caffeine was prevented since caffeine now elutes in the acidic/neutral extract.

The improved detection of ephedrine, in addition to the detection of acetaminophen in the acidic/neutral extract was noted in two separate proficiency samples in which these drugs were missed when screened using the former LLE method.

Screening of postmortem case samples utilizing the SPE method has led to detection of drugs in the acidic/neutral extract such as propofol, topiramate, levetiracetam, acetaminophen, and valproic acid which would have previously been missed. Newer drugs detected in the basic extracts include BZP, TFMPP, 5MeoDIPT, methylone, and MDPV, which could have been missed due to decreased recovery by LLE. In addition heroin, 6-MAM, morphine, and benzoylecgonine were detected in the initial GC/MS screening, as opposed to having to be specifically targeted in other confirmatory assays.

With the constant evolution of designer drugs, it is important for laboratories to respond and adapt accordingly, even though funding for consumables and staff may be limited. By adopting an SPE protocol, the laboratory is now equipped to screen for a variety of tryptamines, as well as the components of the ever-so-popular "bath salts." Additionally, the laboratory has become more efficient due to the reduction in solvent usage and sample preparation time. Other advantages include safety improvements and prevention of errors from multi-step procedures.

More information is obtained from the same sample volume via the dual elution which provides a much more comprehensive screen.

SPE, Comprehensive Screen, Basic Drugs

 ** Presenting Author*

K27 Advanced Automated Library Searching for Compound Identification in Forensic Toxicology Samples

Adrian M. Taylor, PhD, 71 Four Valley Dr, Concord, ON L4K 4V8, CANADA*

After attending this presentation, attendees will learn about the different complimentary screening techniques that can be performed on a hybrid quadrupole linear ion trap to confidently identify targeted and unknown compounds. Attendees will learn about using acquired Tandem Mass Spectrometry (MS/MS) data to search against MS/MS libraries and utilizing a new advanced automated library searching with the capability to dynamically review the collected MS/MS information. Attendees will see that the software substantially improves the "data mining" process and provides an elegant solution to automated processing of the forensic toxicology screening data to confidently identify compounds.

This presentation will impact the forensic science community by demonstrating the advantages of the new automated library-searching approach in improving typical forensic toxicology screening workflows.

Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) utilization in forensic toxicology screening for drugs and drug metabolites has become increasingly popular due to the selectivity, sensitivity, and the speed of LC/MS/MS analysis. MS/MS confirmation with automated searching against available spectral libraries has proven to add a superior level of confidence to the compound identification. One of the key factors of the complete solution for forensic toxicology screening is automation of the library searching with the advanced capability to dynamically review the acquired data. Solutions need to be accurate and robust. The ability to search multiple libraries, create subsets of libraries, adjust and refine search parameters as well as re-search acquired data provides the user with the substantial flexibility. Setting mass tolerance, intensity thresholds, and searching multiple collision energies enhance the data under revision. The ability to search or re-search entire data files or a specific mass spectrum with different parameters can improve overall data quality and throughput. Additionally, reporting tools allow the information to be disseminated to the end user.

Forensic toxicology samples were analyzed using generic sample preparation procedures with two AB SCIEX LC/MS systems: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument. The tandem mass spectrometric measurements were performed using the Collision Energy Spread (CES) feature which ensures the detection of the fragment ions generated in low-, medium-, and high-collision energy regimes. All the collected MS/MS spectra were searched against an AB SCIEX Forensic Drug Spectral Library comprised of over 1,250 compounds. The data processing was performed with the new AB SCIEX prototype library searching tool equipped with two library search algorithms. The accuracy, flexibility, speed, and robustness of the new library searching approach was successfully demonstrated in the processing of the data specifically acquired in different experimental set-ups. The ion trap screening data were collected in three screening workflows that consisted of several looped experiments as follows:

1. Multitargeted Screening:
    a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
    b. Enhanced Product Ion (EPI) dependent scans set to automatically collect MS/MS fragmentation spectra for the targets detected in experiment 1.
2. General Unknown Screening:
    a. Enhanced Mass Spectrum (EMS) monitoring for the detection of the unknown analytes.
    b. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the unknowns detected in experiment 1.
3. Combined multitargeted and unknown screening:
    a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
    b. EMS monitoring for the detection of the unknown analytes.
    c. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the targets identified in experiment 1 and unknowns identified in experiment 2.

The hybrid quadrupole-time-of-flight data collected using a TOF/MS survey scan with IDA-triggering of up to 20 product ion scans was also processed. In all specified cases, both targeted and unknown drugs and metabolites were identified in selected samples with a high level of confidence (based on the values of purity, fit, and reverse fit). Utilization of the new advanced automated library searching with the capability to dynamically review the collected MS/MS information has been demonstrated to substantially improve the "data mining" process and provide an elegant solution to automated processing of the forensic toxicology screening data.

LC/MS/MS, Library Searching, Hybrid Linear Ion Trap

 ** Presenting Author*

K28 An Easy, Fast, and Reliable Workflow to Perform Real Forensic/Toxicological General Unknown Screening

Adrian M. Taylor, PhD, 71 Four Valley Dr, Concord, ON L4K 4V8, CANADA*

After attending this presentation, attendees will learn about a comparative screening workflow that allows the comparison between a sample and control in which significant differences in the sample are automatically extracted resulting in a reduction of several hundred peaks down to identifying only the significant components of the sample. Learning outcomes will include how high resolution accurate mass instrumentation can be successfully used to provide comprehensive and valuable information in identification of unknowns. Currently real General Unknown Screening suffers from complexity of biological matrices, which makes it almost impossible to identify relevant compounds in an easy and fast way. This presentation will present an easy-to-use generic workflow for General Unknown Screening. As an example, Tramadol in urine will be shown to be easily detected with only two injections in a Comparative Screening workflow using a hybrid quadrupole/time-of-flight instrument.

This presentation will impact the forensic science community by showing a fast, confident, and easy-to-use workflow to perform real non-targeted screening. The General Unknown Comparative Screening workflow provides basic sensitivity in Mass Spectrometry (MS) and Tandem Mass Spectrometry (MS/MS) modes for a clear identification of compounds, high resolution to overcome selectivity issues, and mass accuracy to capitalize from the provided resolution.

Method: ekspert™ ultraLC device was coupled to a fast-scanning high resolution MS system providing fast and sensitive MS/MS capabilities. Information-dependant acquisition with dynamic background subtraction and dynamic exclusion triggered 10 MS/MS experiments. The resulting total cycle time ensured that the compounds had more than 10 data points across extracted ion chromatograms (peak width 4 – 5 sec). Total LC runtime was 10 minutes using 6 min gradient (95% de-ionised water to 0% de-ionised water) with Phenomenex Kinetex 2.6µm C18 Column, 100 Å, 50 x 2.1mm column.

Results: The Comparative Screening workflow required two injections; a control injection was followed by the sample injection. The control was a urine sample of approximately comparable matrix to the sample without any drugs. Both data were loaded into PeakView™ software and automatically evaluated by an additional software add-on. All peaks overcoming a defined threshold were evaluated for retention time similarities in both sample and control. Significant differences in the sample due to, for example, absence or lower abundance of the same peak in control were automatically extracted and MS as well as MS/MS information was displayed (defined by second threshold). Thus, a reduction of several hundred peaks down to what is specific to the sample only were identified; Tramadol and its related major metabolites (demethylation). Sensitive MS/MS information can be used for confident identification by automatic searching MS/MS forensic library (1,250 entries). In case of missing conformity of a detected mass with any compound in any library, additional built-in software tools help to identify formulas by accurate mass, isotopic ratio, and sensitive MS/MS information. Finally, potential structures of calculated formula can be verified by fragment-predictive software tools.

Comparative General, LC/MS/MS, Accurate Mass

 ** Presenting Author*

K29 Analysis of the Rate of Decay and Dispersion of Pentobarbital in Soil by Liquid Chromatography/Mass Spectrometry

Chasity D. Suttle, BS, 414 Thomas Jefferson Cir, Madison, TN 37115; and Paul Kline, PhD, 1301 E Main St, Murfreesboro, TN 37132*

The goals of this presentation are to: (1) become familiar with the principles of extracting pharmaceuticals, specifically barbiturates, out of a complex matrix, particularly soil; (2) apply the use of this method to determine the initial concentration of the contaminated area and the focal point of contamination; and, (3) understand the significance of this method in order to determine the time of contamination based on the rate of decay and dispersion dynamics.

This presentation will impact the forensic science community by showing an effective method for extracting barbiturates out of complex matrices, specifically soil, is necessary for analytical analysis and has significant impact in the field of forensics and environmental science.

A method for detecting the barbiturate pentobarbital in soil previously developed was utilized to determine its application to the decay rate and dispersion rate of pentobarbital and similar barbituric acid derivatives in soil.

Pentobarbital is a pyrimidine derivative in a class of organic drugs called barbiturates. Several thousand derivatives of barbituric acid have been synthesized with far-reaching effects and flexible durations of action. Duration of action refers to the length of time the drug affects the target system, and in the case of humans or animals, it is the Central Nervous System (CNS). Pentobarbital is categorized as a fast-intermediate sedative-hypnotic drug. Barbiturates are highly stable organic compounds that are released into the environment via multiple pathways. Barbiturates have been extensively used throughout the United States. Euthanized animals are a growing contamination source in addition to the contribution of barbiturates from a wide array of pharmaceutical use, misuse, and abuse.

The method was developed to quantify the rate of decay of pentobarbital in contaminated soil. Pentobarbital in addition to other barbituric acid derivatives were extracted from separate soil samples, each separately spiked with the respective barbituric acid derivative. Clean-up procedures involved centrifugation, reverse-phase Solid Phase Extraction (SPE), microfiltration, and lastly, analysis by Liquid Chromatography/Mass Spectrometry (LC/MS). Concentration determination and recovery were determined utilizing a deuterated isotope method, Pentobarbital-D5, and an internal standard method. Satisfactory recoveries of the barbituric acid derivatives indicate this is an effective method for analysis and detection. Further, pre-concentration via solid phase extraction allowed for 0.001mg of barbituric acid derivative per five grams of soil (200 parts per billion) to be detectable at limits of quantification using LC/MS. This method can be suitable for larger quantities of soil and applicable for a wide range of soil types.

The development of extraction methods for pharmaceuticals out of soil has multiple applications in the scientific community. Additionally, the application of this extraction method as to the determination of the source of contamination, date of contamination, and amount of contamination has significant impact in the field of forensics.

Barbiturate, Soil, Decay

 ** Presenting Author*

K30 Method Development and Validation of Dimethylamylamine (DMAA, Methylhexanamine) by Gas Chromatography-Mass Spectrometry

Brittany D. Pasierb, BS, 700 Lower State Rd, Bldg 25, Unit A8, North Wales, PA 19454; Jillian K. Yeakel, MS, 105 Revere Dr, Harleysville, PA 19438; Celeste Metzger, MS, 2300 Stratford Ave, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will understand how a method was developed and validated for the detection of Dimethylamylamine (DMAA, methylhexanamine) in nutritional supplements and urine samples using Gas Chromatography/Mass Spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a method for the detection of the emerging drug of abuse, and banned stimulant drug DMAA, and raise awareness of its use and presence in over-the-counter supplements and "legal highs."

Marketed as a nasal decongestant in the 1940s, DMAA, also known as methylhexanamine, currently has no recognized medical use. In 2006, the compound began to be added to nutritional supplements such as weight-loss preparations and pre-exercise pills. DMAA is a central nervous system stimulant which in excess produces effects similar to, but not as intense as, amphetamine. Combined with its availability and perceived low toxicity, DMAA is highly susceptible to abuse. DMAA use was recently associated with the death of two United States soldiers, was added to the World Anti-Doping Agency's Prohibited List, and is currently restricted in several countries.

As the compound is restricted in a number of countries worldwide, it is important to have an established, reliable method for its detection. Quantifying the concentration of DMAA in popular nutritional supplements will also benefit the forensic science community to the extent of determining just how potent and dangerous these supplements are to the public.

The objective of this research was to develop and validate a method for the detection of methylhexanamine in nutritional supplements and urine samples using GC/MS.

The analysis of DMAA is more challenging due to its two diastereomers and reactive primary amine group. After reconstitution studies with methanol, acetonitrile, isopropanol, ethyl acetate, and dichloromethane, DMAA was determined to be insufficiently stable for proper analysis on the GC/MS without derivatization. Following time and temperature studies, a successful derivatization method using 4-carbethoxyhexafluorobutyrl chloride (4-CB) that produced the two expected DMAA chromatographic peaks was developed. Extracts were derivatized by addition of 4-CB with ethyl acetate at 70°C for 20 min. Detection was performed by selected ion monitoring by GC/MS, using amphetamine-d5 as an internal standard.

A Liquid-Liquid Extraction (LLE) technique was used to isolate DMAA using concentrated ammonium hydroxide and chloroform/isopropanol/n-heptane (50:17:33) as the organic extraction solvent. The solvent was removed and evaporated at 33°C under a stream of nitrogen gas. Successful calibration curves have been established across the concentration range 1 – 20µg/mL. The curves generated acceptable r^2 values of 0.997 for DMAA peak 1 and 0.998 for DMAA peak 2. Successful calibration curves have also been established across the concentration range of 10 – 100ng/mL. These curves generated acceptable r^2 values of 0.994 and 0.993 for DMAA peak 1 and DMAA peak 2 respectively. In addition, the limit of detection and limit of quantitation were both preliminarily determined to be better than 10ng/mL, which is determined to be acceptable for both the analysis of solid dosage materials and expected concentrations in biological fluids.

The method is being applied to analysis of DMAA in nutritional supplements containing the drug and to biological samples. The presentation will also review the pharmacology of DMAA and reported adverse effects.

DMAA, Supplements, GC/MS

 ** Presenting Author*

K31 Profiling of Inhalants and Common Blood Alcohol Interferences Using Headspace- GC/FID and Headspace-GC/MS

Amanda Rigdon, BS, and Jonathan Edelman, BS, 110 Benner Cir, Bellefonte, PA 16823; Frank Dorman, PhD, 107 Whitmore Labs, University Park, PA 16802; and Kristi Sellers, BS, and Jim Whitford, BS, 110 Benner Cir, Bellefonte, PA 16823*

WITHDRAWN

K32 A Proposed Means for the Detection and Quantification of Bath Salts From Blood

Sarah E. Sims, BS, 144 Interstate 45 N, #1216, Huntsville, TX 77320; and Chris Heartsill, BS, and Elizabeth L. Todd, PhD, 2355 N Stemmons Fwy, Dallas, TX 75207*

After attending this presentation, attendees will understand the potential for Gas Chromatography/Flame Ionization Detection (GC/FID) use as a means of detecting and quantifying synthetic cathinones (bath salts). In addition, attendees will be aware of an assay that successfully extracts bath salts from blood samples. Finally, attendees will be aware of the stability of these extracts over a short period of time.

This presentation will impact the forensic science community by demonstrating a method for the extraction, detection, and quantification of a subset of novel drugs of abuse, specifically bath salts. As these drugs are quickly being banned at both the state and federal level, it is critical that laboratories develop appropriate assays in a timely fashion. Adoption of the method described in this study, rather than in-house method development, would allow laboratories to more quickly add bath salts to the drugs of abuse which they can report. In addition, the stability study performed on the extracts will provide valuable information for validation studies that must be performed in each laboratory.

Drug abusers often attempt to circumvent controlled substance legislation by manufacturing and using novel compounds. Recently, synthetic derivatives of the natural alkaloid cathinone, more commonly known as bath salts, have been used by drug abusers seeking legal alternatives to more common drugs of abuse such as amphetamines. Because these compounds are novel, few laboratories have validated methodologies for the extraction, detection, and quantification of these compounds. This study proposes a method for this purpose.

This study utilized a basic alkaline extraction procedure to extract methcathinone, mephedrone, pentedrone, 4-methylethcathinone (4-MEC), methylone, α-pyrrolidinopentiophenone (α-PVP), butylone, and methylenedioxypyrovalerone (MDPV) from spiked blood samples. These extracts were then subjected to both GC/FID and Gas Chromatography/Mass Spectrometry (GC/MS). Retention times were noted for the samples on GC/FID and compared for possible co-elution. The compounds separated well with the exception of the co-elution of α-PVP and butylone on channel one. However, there was clear separation on the second channel between these two compounds, allowing all compounds to be combined into a single master mix for curve generation. GC/MS results were used to verify the identity of the compounds. Both GC/FID and GC/MS showed a good response from all compounds, demonstrating that the methods used on the instruments were appropriate for detection of bath salts. Calibrators were then created for each compound at 0.02mg/L, 0.05mg/L, 0.15mg/L, 0.50mg/L, and 1.00mg/L. The calibrators were run on the GC/FID and the area ratio compared to the internal standard alphaprodine was used to create standard curves that could be used to calculate the concentration of each of the compounds. These curves were linear over the 0.02mg/L – 1.00mg/L range using all five points. Each curve achieved a minimum R^2 value of 0.995.

Following the establishment of curves on the GC/FID, a stability study was performed on the extracts to determine the stability of each compound at each concentration over a period of one week. The extracts were run each day for a week and their concentrations were charted to determine any change over time. The overall trend indicates that the compounds begin to degrade at concentrations greater than or equal to 0.15mg/L after a 24-hr period.

Samples from cases in which bath salts were previously detected were then re-extracted to determine for which matrices the assay was suitable. Samples included antemortem and postmortem blood, urine, muscle tissue, and vitreous fluid, all of which are common sample types available in forensic analysis. The compounds of interest were both detected and quantified via the assay, demonstrating the suitability of these matrices.

Synthetic Cathinones, GC-FID, Quantification

 ** Presenting Author*

K33 Capillary Electrophoresis and Capillary Electrochromatography Mass Spectrometry for Charged and Neutral Drug Detection

Hanzhuo Fu, BS, Florida International Univ, Dept of Chemistry, 11200 SW 8th St, Miami, FL 33199; and Bruce R. McCord, PhD, Dept of Chemistry, Florida International Univ, University Park, Miami, FL 33199*

WITHDRAWN

 ** Presenting Author*

K34 Comprehensive Toxicological Examination in a Case Involving Alleged Use of Bath Salts: The Importance of a Negative Result

Barry K. Logan, PhD, and Sherri L. Kacinko, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to define the appropriate scope of testing for alleged "Bath Salts" intoxication cases, and appreciate that negative toxicology findings can only be properly interpreted when the scope of testing is understood.

This presentation will impact the forensic science community by providing an approach to rule out drug intoxication with emerging hallucinogenic drugs as part of a complex death investigation. It will also emphasize for the public and the press the importance of waiting for completion of toxicological testing before making assumptions about the role for intoxicants in criminal or death investigations.

In the assessment of crimes of extreme violence or where there is evidence of delusional or psychotic behavior, increasingly suspicion falls on the possible role of emerging designer stimulants and hallucinogens. Similar behaviors, however, can result from mental illness, schizophrenia, or even intoxication with traditional recreational drugs or drugs of abuse.

The importance of a structured escalating set of analytical protocols for these types of investigations cannot be overstated. Once routine toxicological analysis rules out traditional recreation drugs of abuse, the routine testing can begin. A recent case involving a violent assault provides a good example of how the application of routine tests can be supplemented by tests for additional drugs relevant to the fact pattern of the investigation

In this case, routine drug testing did not reveal the presence of any drug which could explain the extremely violent nature of the attack. Further testing was needed. Additional testing for the common "bath salts" compounds, MDPV, mephedrone and methylone, was performed. In addition a comprehensive screen for a wide variety of both traditional and emerging stimulant and hallucinatory drugs (67 in total) was performed using Liquid Chromatography-Time Of Flight-Mass Spectrometry (LC-TOF/MS). This included tryptamines, designer phenethylamines, other stimulants, cathinones, traditional stimulant drugs including cocaine and its metabolites and amphetamines, methoxy derivatives of phenethylamines, LSD, mescaline, psilocin, and bufotenin. Additional gas chromatography-mass spectrometric analysis was performed and compared to additional libraries containing over 10,000 known designer drugs and their analogs. The chromatographic data were further scrutinized for the presence of unidentified peaks; however, none were found.

Further testing for a comprehensive scope of emerging synthetic cannabinoid compounds was performed also. These drugs have been linked to adverse effects including paranoia, anxiety, psychosis, and violent assaults. A total of 20 synthetic cannabinoid drugs were tested for, including JWH-018, JWH-073, JWH-250, AM-2201, JWH-200, RCS-4, JWH-210, AM-694, JWH-122, JWH-081, JWH-019, RCS-8, JWH-203, JWH-022, JWH-018 5-chloropentyl, UR-144, XLR-11, AM-2233, AM-1248, and A-796,260. None were detected in the blood samples from the case.

Some of these emerging designer drugs, especially in the 2C series and derivatives of MDPV, as well as traditional hallucinogens like LSD and psilocin, are known to have limited stability in biological fluids, so appropriate collection and storage conditions are important in investigation of some of these more labile compounds. Consequently, their use cannot be ruled out even when toxicological tests are negative, and this places more of a burden on the results from a thorough investigation of the scene and history and circumstantial evidence of drug residue and paraphernalia when they exist.

In this case, comprehensive testing of available blood samples starting with routine analytical tests, and the addition of more specialized tests targeted to the compounds of concern, showed the presence of no drugs. No physical evidence from the scene or from the history revealed the presence of specific esoteric drugs that might have been ingested. The reasonable conclusion when the history, scene, autopsy, and toxicological evidence is considered in this case is that designer drug use was not a factor in this case.

Bath Salts, Designer Drugs, Drugs of Abuse

 ** Presenting Author*

K35 Death by "Legal Psychedelic Piperidines and Phenethylamines": Postmortem Tissue Distribution of Desoxypipradrol (2-DPMP) and 4-Chloro-2,5- Dimethoxyamphetamine (DOC)

Eric S. Lavins, BS, Cuyahoga County MEO, 11001 Cedar Ave, Cleveland, OH 44139; Mary Pietrangelo, MD, 1160 Oakwood Ln, Saint Clair, MI 48079; Kevin G. Shanks, MS, 2265 Executive Dr, Indianapolis, IN 46241; Cassandra E. Cushman, West Virginia Univ, Morgantown, WV 26505; Sofalvi Szabolcs, MS, and; Joseph A. Felo, DO, Cuyahoga County MEO, 11001 Cedar Ave, Cleveland, OH 44106; John F. Wyman, PhD, Cuyahoga County Regional Forensic, Toxicology Section, 11001 Cedar Ave, Cleveland, OH 44106; and Thomas P. Gilson, MD, Cuyahoga County MEO, 11001 Cedar Ave, Cleveland, OH 44106*

After attending this presentation, attendees will have a better understanding of the piperidine and amphetamine class designer drugs desoxypipradrol (2-DPMP) and 4-chloro-2,5-dimethoxyamphetamine (DOC) and their concentrations in postmortem matrices.

This presentation will impact the forensic science community by informing forensic professionals on new abuse trends for amphetamines and designer drugs, particularly in our youth. It adds to the relatively sparsely published data concerning the potential toxicity of these stimulant drugs and provides a comprehensive approach to extraction, detection, and quantification of these substances.

These type of compounds have recently achieved "epidemic" status for abuse by young people. 2-DPMP exhibits a cocaine-like binding profile while DOC is a long-acting agonist of serotonin receptors; the fluoroamphetamines stimulate release and prevent reuptake of dopamine, serotonin, and norepinephrine.

Toxicities observed are similar to amphetamine toxicity: tachycardia, nausea, hypertension (vasoconstriction), insomnia, hyperthermia, mydriasis, panic attack, and seizures with the added predominant neuropsychiatric features of hallucinations, paranoia, and agitation.

A 30-year-old White male called a friend and advised him he had been snorting "DOC," 4-chloro-2,5-dimethoxyamphetamine, and was "tripping" and needed assistance. EMS and police were called and found the individual lying face down, breathing, but unresponsive, convulsing, extremely warm to the touch, and sweating heavily. He was conveyed to the hospital with an initial diagnosis of an acute drug overdose. The decedent had a history of selling, manufacturing, and using illegal drugs. All indications were that the overdose occurred within the last three hours. The individual died 42 hrs later at the hospital. A collection of narcotics and drug paraphernalia were confiscated from the residence as evidence and later submitted for analysis.

An autopsy was performed at the Cuyahoga County Medical Examiner's Office. Autopsy findings included dilated cardiomyopathy with a 460-gram heart, cerebral edema, and edematous lungs. Heart and femoral blood, vitreous humor, bile, liver, brain (medulla), and gastric were submitted for a comprehensive toxicology analysis.

The heart blood was positive for amphetamine 0.058mg/L, methamphetamine 0.170mg/L, fentanyl 2.0ng/mL; norfentanyl 0.44ng/mL, acetaminophen, atropine, caffeine, cotinine, lidocaine, and nicotine. The femoral blood was not sufficient in volume for analysis. No antemortem admission blood samples were available for subsequent analysis.

Because of the decedent's drug history, further testing was performed to determine the presence of other possible phenethylamine and amphetamine class drugs. Samples were extracted at a basic pH into ethyl acetate. 2-DPMP, DOC, and the fluoroamphetamines were separated and detected by an Agilent GC/MS-EI in full scan mode with a Restek-DB5 capillary column.

Further confirmatory testing was performed at AIT Laboratories, Indianapolis, IN, for the 2-DPMP, DOC, and fluoroamphetamines. Specimens were extracted at a basic pH into n-butyl chloride. Separation and detection was completed by a Waters Acquity UPLC coupled to a Waters LCT Premier XE TOF mass spectrometer as well as a Waters Acquity UPLC coupled to a Waters Tandem Quadrupole Detector (TQD). The analytical column for both analyses was a Waters BEH C18, 2.1 x 100mm, 1.7µm particle size.

The concentrations for the subsequent testing are as follows: desoxypipradrol (2-DPMP) concentrations (mg/L) were 0.283, heart blood; 0.236, vitreous humor; 1.98mg/kg, liver; 0.817mg/kg, brain (medulla); >1.0, bile, and negative, in the gastric.

4-chloro-2,5-dimethoxy amphetamine (DOC) concentrations (mg/L) were 0.466, heart blood; 0.380, vitreous humor; 1.40mg/kg, liver; 1.09mg/kg, brain (medulla); and 2.04 in the bile. Fluoroamphetamine and fluoromethamphetamine were qualitatively present in all the specimens.

2-DPMP and DOC was found to be distributed among multiple matrices with values ranging from 0.236 to >1.0mg/L for 2-DPMP and 0.380 to 2.04mg/L for DOC. Tissues responsible for detoxification/excretion had higher concentrations of the drugs. 2-DPMP, DOC, and the fluoroamphetamines were present in all tissues analyzed except gastric.

Drug chemistry results from submitted drug and drug paraphernalia exhibits were found to contain the following: 2-fluoromethamphetamine; alprazolam; 2-(1-pyrrolidinyl)-(4-methylphenyl)-1-propanone (MPPP); methamphetamine; dimethyltryptamine (DMT); psilocin; cannabis; fluoromethamphetamine; 4-chloro-2,5-dimethoxyamphetamine (DOC); phencyclidine; and, lysergic acid.

 ** Presenting Author*

This case was consistent with the suspicion that this was an acute drug exposure. The cause of death was ruled toxic metabolic encephalopathy due to mixed drug intoxication. The manner of death was ruled as accidental.

Desoxypipradrol (2-DPMP), 2,5-dimethoxyamphetamine (DOC), Designer Drugs

 ** Presenting Author*

K36 *In Vitro* Formation of Acetylmorphine From Morphine and Aspirin in Gastric Contents and Water

Claire K. Kaspar, BS, Cuyahoga County MEO, 11001 Cedar Ave, Cleveland, OH 44106; John F. Wyman, PhD, Cuyahoga Co Regional Forensic, Toxicology Section, 11001 Cedar Ave, Cleveland, OH 44106; Cassandra E. Cushman, West Virginia Univ, Morgantown, WV 26505; Leon Glass, PhD, 3978 Mack Rd, Fairfield, OH 45014; and Eric S. Lavins, BS, and Thomas P. Gilson, MD, Cuyahoga County MEO, 11001 Cedar Ave, Cleveland, OH 44106*

After attending this presentation, attendees will understand that it is possible to form acetylmorphine *in vitro* by incubating human gastric contents or Deionized (DI) water with morphine and aspirin.

This presentation will impact the forensic science community by suggesting that detection of 6-acetylmorphine (6-AM) may no longer solely be an indicator of heroin use.

Forensic toxicologists across the world have considered detection of 6-AM to be definitive evidence of heroin use. 6-AM was detected in an 85-year-old female with a history of a witnessed arrest in bed at a nursing home. The decedent was under hospice care for failure to thrive, and had a history of multiple strokes, syncope, hyperkalemia, osteoporosis, anemia, and osteomyelitis. There was no history of illicit drug use and the decedent was prescribed morphine sulfate elixir (Roxinol®). Whether she was taking aspirin (acetylsalicylic acid) was not recorded and salicylates were not detected by colorimetry. The manner of death was natural and the cause of death was ruled as bronchopneumonia due to hypertensive atherosclerotic cardiovascular disease with remote myocardial and cerebral infarcts.

What about the 6-AM? Is it possible that an individual may be taking morphine (pain management) and aspirin (anticoagulant), and the aspirin may acetylate morphine to produce acetylmorphine? In the present study, the possibility of formation of acetylmorphine when morphine is mixed in solution with aspirin was investigated.

Two opioid negative, postmortem gastric specimens were selected for this study, along with morphine sulfate-Extended Release (ER) tablets (15mg) and coated-aspirin tablets (325mg). Morphine and aspirin tablets were placed into 50mL samples of the two separate gastric specimens, as well as deionized water. The three morphine/aspirin solutions were incubated at 37°C for increasing lengths of time. A separate experiment was run in gastric contents using 15mg morphine sulfate powder in lieu of morphine extended-release tablets. One milliliter aliquots were taken from all samples at 10 min intervals up to one hour, and then at 90 min, two hours, and ultimately 26 hrs. Aliquots were extracted using a previously published UCT solid phase opiate procedure, and analyzed by GC/MS in SIM mode.

Acetylmorphine was detected in all of the samples containing morphine and aspirin in combination. Levels of acetylmorphine were greater in gastric contents than in DI water during the same incubation period. After 120 min, the 6-AM concentrations for the samples containing aspirin and an ER tablet were 21ng/mL and 25ng/mL in the gastric solutions, compared to 7ng/mL in water. After 26 hrs at room temperature, the gastric concentrations were 124ng/mL and 121ng/mL, and in water 27ng/mL. The increase in concentration of acetylmorphine in gastric was linear (R^2 = 0.99 and 0.98), while formation in water was non-linear (R^2 = 0.63). The results for morphine sulfate powder were essentially identical to those observed for ER tablets. The initial pH of the two gastric samples were 4.74 and 5.27, respectively; following the addition of the morphine/aspirin tablets and two hours incubation, final pH values were 3.86 and 3.92. The final pH of the water solution was 2.88. This study demonstrates that it is possible to form acetylmorphine *in vitro* by combining morphine and aspirin tablets in both postmortem gastric contents and deionized water. The compound produced in this study was identified as 6-AM by GC/MS. Further investigation must be done to determine whether the compound is actually 6-acetylmorphine, 3-acetylmorphine, or a mixture of the two compounds.

Does acetylmorphine form *in vivo*? In addition to the case described above, 10,602 specimens were assayed for opioids by a pain management laboratory using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Three cases containing acetylmorphine were found to be inconsistent with heroin usage. A single specimen was listed as having a prescription for morphine and contained codeine, morphine, and 6-AM; the other two specimens contained 6-AM but not morphine or codeine. Although *in vitro* formation of acetylmorphine has been demonstrated, these data indicate that *in vivo* formation from the co-administration of aspirin and morphine is unlikely to occur. This may be attributed to inconsistencies in elimination half-lives; half-lives are 13 – 20 minutes and 1.3 – 6.7 hours for aspirin and morphine, respectively.

Morphine, Acetylsalicylic Acid, Acetylmorphine

 ** Presenting Author*

K37 An Investigation of the Binding of Benzodiazepines to Human Serum Albumin and the Effect on Quantitation in Blood Samples

Kristina M. McNerney, BS, Cedar Crest Coll, 100 College Dr, Box 956, Allentown, PA 18104; and Marianne E. Staretz, PhD, Cedar Crest Coll, Dept of Chemical & Physical Sc, 100 College Dr, Allentown, PA 18104*

After attending this presentation, attendees will understand how the binding of benzodiazepines to Human Serum Albumin (HSA) can affect the quantitation of benzodiazepines in blood samples. Attendees will also be made aware of how the varying binding affinities of different benzodiazepines for human serum albumin can affect quantitation within specific sample preparation methods.

This presentation will impact the forensic science community by providing further pharmacological/toxicological information on benzodiazepines, a class of drug that is commonly used therapeutically and is increasingly being abused in social settings.

Benzodiazepines are commonly prescribed central nervous system depressants which are found in a wide variety of different medications, from sedatives and hypnotics to amnesiatics and anticonvulsants. Benzodiazepines are increasingly being used as recreational drugs, often in combination with other drugs such as opiates and alcohol. HSA is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and characterize the binding affinities and other important binding characteristics. In a preliminary investigation, the binding affinities and other binding characteristics for alrazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam to HSA were tabulated. The binding constants of the nine benzodiazepines ranged from $1.14x10^{2}$M for diazepam, having the lowest binding affinity, to $8.05x10^{6}$M for flunitrazepam, with the highest binding affinity. The binding of these drugs to HSA and the binding affinity of each benzodiazepine derivative may affect the quantitation of these drugs in blood. In the current research, different preparation methods were utilized on samples spiked with known amounts of benzodiazepine. Quantitation was accomplished using an Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method with Multiple Reaction Monitoring (MRM) which utilized a C18 column and isocratic elution with 0.1% formic acid in methanol (60%) and 0.1% formic acid (40%) at a total flow rate of 0.3500mL/min. The temperature range was 40°C – 95°C. Within a preparation method, the effect of differing binding affinities on the quantitation was studied. In a dilute and shoot method, flunitrazepam, a benzodiazepine which was shown to have a high binding affinity for albumin, showed a significant difference when quantitated in samples containing human serum albumin compared to samples without human serum albumin. Samples containing HSA had calculated concentrations that were 31% – 50% lower than samples without HSA. Diazepam, which was shown to have a lower binding affinity for albumin, also showed a significant difference when quantitated in samples containing HSA compared to those without. Samples containing HSA had a calculated concentration that was 40% – 60% lower than samples without HSA. Other preparation methods were used; a comparison of these results will also be presented.

Benzodiazepines, Human Serum Albumin, Quantitation

 ** Presenting Author*

K38 Intraosseous Fluid as Alternative Biological Specimen in Postmortem Toxicology Case Evaluations

Justin A. Volk, OCME, and Chinyere M. Williams, BS, OCME, 850 Bryant St, San Francisco, CA 94103; Ellen Moffatt, MD, San Francisco OCME, 850 Bryant St, N Ter, San Francisco, CA 94103; Alan H. Wu, PhD, Rm 2M27, 1001 Potrero Ave, San Francisco General Hospital, San Francisco, CA 94110; Amy P. Hart, MD, San Francisco OCME, 850 Bryant St, N Ter, San Francisco, CA 94103; and Nikolas P. Lemos, PhD*, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103*

After attending this presentation, attendees will understand the usefulness and value of Intraosseous Fluid (IOF) as a possible toxicologic specimen in postmortem cases of medicolegal interest.

This presentation will impact the forensic science community by providing valuable information on the collection of IOF during autopsy as well as its analysis by Enzyme Linked Immunosorbent Assay (ELISA) for several commonly encountered drugs in postmortem toxicologic evaluations.

In San Francisco, sudden, unexpected, or violent deaths are investigated by the Office of the Chief Medical Examiner. Autopsies are performed and biological specimens are collected for laboratory tests. Such specimens commonly include blood (central/cardiac and peripheral), urine, liver, and vitreous humor. Blood and vitreous humor are routinely screened for ethanol and related compounds by headspace gas chromatography equipped with flame ionization detection. Blood (central/cardiac) and urine specimens are further screened by ELISA for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, fentanyl, methadone, opiates, oxycodone, phencyclidine, and tricyclic antidepressants using commercially available ELISA kits and by Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode for over 100 common drugs and metabolites. Confirmations and quantitations are normally carried out in blood (peripheral) and urine as necessary to complete the toxicologic evaluations.

After evisceration, IOF specimens were collected from 30 decedents and from four different parts of the body (right tibia, left tibia, right humerus, and left humerus) together with other standard autopsy specimens. IOF specimens were collected using donated EZ-IO® intraosseous systems by Vidacare in 10mL syringes with continuous suction provided by holding back the commercially available plungers with mosquito forceps. IOF specimens were then transferred into clean gray top test tubes. All specimens were refrigerated until the time of analysis.

Blood (central/cardiac) and urine specimens were screened by ELISA per the Office's standard testing protocol. Additionally, IOF specimens were screened by ELISA using commercially available kits donated by Venture Labs, Inc. The kits used in the IOF experiments were designed and validated for blood analyses and the analysis took place using blood drug cut-offs. The ELISA drug screening results in IOF specimens for cocaine, amphetamine, methamphetamine, opiates, oxycodone, methadone, tricyclic antidepressants, fentanyl, and phencyclidine closely correlated with the ELISA drug screening results in blood. Correlation between blood ELISA and IOF ELISA results was 100% for cocaine, opiates, methadone, phencyclidine, and fentanyl, over 90% for tricyclic antidepressants (91%), and oxycodone (93%) but dropped to 89% for cannabinoids, 75% for methamphetamine, and 69% for amphetamine. Additionally, it appears that body origin of the IOF specimen may contribute to the correlation between IOF ELISA results and blood ELISA results since IOF from the left humerus and the left tibia showed slightly higher correlation to the blood ELISA results (93% and 93%, respectively) than those from the right humerus (91%) and the right tibia (86%).

Further studies are needed to fully investigate the potential of IOF in postmortem toxicology including quantitation of drugs in IOF and evaluation of this fluid's susceptibility, if any, to postmortem redistribution and interval, two issues that often arise when dealing with drug concentrations in postmortem blood specimens. This limited study suggests that IOF specimens appear to be relatively easy to collect at autopsy using commercially available collection devices, permit the collection of enough specimen volume for ELISA testing and appear to closely mimic blood ELISA drug screening results. For these reasons, intraosseous fluid should be considered as an alternative biological specimen by forensic pathologists, coroners, medical examiners, and forensic toxicologists for drug screening by ELISA in postmortem toxicology investigations.

Intraosseous Fluid, Toxicology, ELISA

 ** Presenting Author*

K39 Quantitative Analysis of Endogenous Levels of Gamma-Hydroxybutyric Acid (GHB) in Hair Samples Using Different Extraction Techniques

Megan E. Crowley, BS, 213 Race St, Macungie, PA 18062; Marianne E. Staretz, PhD, Cedar Crest Coll, Dept of Chemical & Physical Sc, 100 College Dr, Allentown, PA 18104; and Michelle Schmidt, MS, Cedar Crest Coll, 100 College Dr, Allentown,PA, PA 18104*

After attending this presentation, attendees will understand how a series of freeze/thaw cycles can be used to extract Gamma-Hydroxybutyric Acid (GHB) from human hair samples. Attendees will learn how freeze/thaw cycles can be a quick and reliable method for the analysis of hair samples from individuals suspected of illicit drug use.

This presentation will impact the forensic science community by providing a quick extraction method to use for hair analysis of GHB. It is thought that in the future this extraction method can be used to extract other drugs from human hair samples. This extraction method will help to decrease the analysis time of hair samples when they are encountered in the forensic science community.

GHB is a short chain fatty acid that was originally used for medicinal purposes such as the treatment of alcoholism and clinical depression. In 2005, GHB was approved under the trade name Xyrem® to treat narcolepsy and cataplexy. Despite its approved medicinal applications, GHB is primarily associated with being a recreational drug and drug of abuse. Within the forensic science community, GHB is most often observed in cases of drug-facilitated sexual assaults. Due to its synergistic effects with alcohol and quick onset of amnesia, GHB is an attractive drug of abuse for criminals attempting sexual assault.

The half-life of GHB in a healthy individual is only 20 – 53 min, which leaves a narrow and abrupt window for detection. Urinalysis, a conventional technique for detecting illicit drug use, is difficult to use because GHB is undetectable in urine within twelve hours after ingestion. This presents an additional obstacle for forensic scientists because many sexual assaults are not reported within this twelve-hour time window. Hair analysis of GHB may prove useful in the detection of drug-facilitated assaults involving GHB by allowing for a longer detection window. Most current hair extraction techniques are time-consuming and require a significant amount of sample preparation. Extraction using freeze/thaw cycles requires less time and less sample preparation. The freeze/thaw cycles consist of first washing the hair and then placing the hair into ethanol, which is used to extract the GHB from the hair. Once the hair is placed into the ethanol, the samples are then placed in liquid nitrogen until the ethanol is frozen (about 30 sec). The sample is then left to thaw, which usually takes about 2 min. This process is repeated for a total of five times. Once the sample is dried down, it is then derivitized and reconstituted in acetonitrile and then analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Quantitation of GHB extracted from hair was accomplished using a pulsed-splitless GC/MS method, which had a pulse pressure of 30.0psi that was held for 1.75 min. This method was determined to be sensitive and robust for analysis of GHB.

The current research indicates that freeze/thaw cycles are comparable to other extraction techniques. The freeze/thaw extraction has worked on multiple types of hair and has allowed for efficient detection and quantitation of endogenous levels of GHB in human hair samples. This method has been shown to detect endogenous levels of GHB at 0.1ng/mg of hair. This method should also prove useful in the analysis of hair samples where illicit GHB use is suspected.

Endogenous GHB, Hair Analysis, Freeze Thaw

 ** Presenting Author*

K40 Development and Validation of an LC/MS/MS Method for the Detection of the Metabolites of JWH-018 and JWH-073 in Human Urine

Moonhee Jang, PhD, National Forensic Service, 331-1 Sinwol-7-dong, Yangcheon-gu, Seoul, Korea 158-707, KOREA; Wonkyung Yang, PhD, 139 Jyangno Yangcheon-Gu, Seoul, 158-707, KOREA; Eunmi Kim, PhD, 139 Jiyangno Yangcheon-gu, Seoul, KOREA; and Hee-Sun Chung, PhD, 1402-703 Mokdong Apt, Shunjung-dong, Yangchon-ku, Seoul, 158-774, KOREA*

After attending this presentation, attendees will learn the analytical method for the determination of major metabolites of JWH-018 and JWH-073 in human urine, and the quantification results of authentic urine samples.

This presentation will impact the forensic science community by presenting the fully validated analytical method for the detection of the main metabolites of new designer drugs, JWH-018 and JWH-073, in urine.

Due to their cannabis-like effect, synthetic cannabinoids have attracted much public attention since 2008. Thus, elucidation of the metabolic pattern as well as detection of the intake of these drugs has been of major concern. In the present study, a sensitive and reliable analytical method was established and validated for the simultaneous determination of the metabolites of JWH-018 and JWH-073 in human urine. For the routine screening in urine, (ω) and (ω-1)-hydroxyl, carboxyl, and hydroxyindole metabolites were selected as target drug metabolites. The samples were prepared by solid-phase extraction and analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The LODs were 0.025ng/mL or 0.1ng/mL and the LOQs were 2.5ng/mL for all analytes. The results of the intra- and inter-day precision and accuracy were satisfactory: <10% for precision and within ±10% for accuracy at low (2.5ng/mL) and high (75ng/mL) concentrations. In this analytical method, no significant matrix effect was observed and high recoveries for all metabolites were achieved. The described method was applied to 52 authentic urine samples suspicious of JWH-018 or JWH-073 abuse and the quantification results among samples were compared. Twenty-one of the samples (40%) were found positive for at least one metabolite of JWH-018 or JWH-073. Carboxylated metabolite of JWH-073 was detected in all analyzed samples, which could be due to the metabolism of JWH-018 in humans. However, (ω) or (ω-1)-hydroxyl metabolite of JWH-073 was detected in only 12 samples. And only a small amount of these metabolites was detected compared with JWH-018 metabolites in most of the analyzed samples. In 14 samples of a total of 21 samples, (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolites, with a mean concentration ranging from 2.9 to 671.2ng/mL; however, in the rest of the samples, the relative concentration of (ω-1) hydroxyl metabolite of JWH-018 was very low (<LOQ-23.4ng/mL) compared with that of the most abundant metabolite in the respective sample. It can be assumed that herbal mixtures used by the suspects contain JWH-073 as an impurity. 6-hydroxyindole metabolite of JWH-018 was detected in samples where (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolite. Similarly, 6-hydroxyindole metabolite of JWH-073 was detected in only two samples which contain (ω-1) hydroxyl metabolite of JWH-073 with a concentration of more than LOQ. These results suggest that at least three metabolites including (ω) and (ω-1)-hydroxyl and carboxyl metabolites should be simultaneously monitored to prove intake of JWH-018 or JWH-073. The variation in the concentrations of detected metabolites could be due to the dosage of the drug and time intervals between the use of the drug and urine collection. However, the absence of detailed information such as dosage, content of synthetic cannabinoids in herbal mixture, and urine collection time makes it difficult to interpret the variation of concentrations between metabolites in the pharmacokinetic aspects. Thus, further study for the estimation of the profiles of metabolite concentrations after JWH-018 or JWH-073 intake versus time will be essential. The developed analytical method will be useful for confirmation and quantification of the metabolites of JWH-018 and JWH-073 in urine in the field of forensic toxicology.

JWH-018 & JWH-073, Metabolite, LC/MS/MS

 ** Presenting Author*

K41 Carisoprodol and Meprobamate Incidence in DUID Cases in the City and County of San Francisco

Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103; and Eric A. Ingle, BA, Cecilia O. Medina, BS, Glenda M. Easterling, BS, Pavlos Karamanidis, BS, and Chinyere M. Williams, BS, OCME, 850 Bryant St, San Francisco, CA 94103*

After attending this presentation, attendees will understand the reasons carisoprodol and meprobamate can impair one's ability to safely operate a vehicle, the incidence of these two drugs in DUID cases during a three-year period (January 1, 2009 to December 31, 2011), the blood concentrations measured in these drivers, and the identity of other drugs found in the blood of these drivers.

This presentation will impact the forensic science community by adding to the existing body of literature information regarding driving behavior, drivers' symptomology, blood concentrations, and other drugs found present in the blood of drivers driving under the influence of carisoprodol and meprobamate.

Carisoprodol and meprobamate are medications that are available only by prescription in the U.S. Carisoprodol is a muscle relaxant and meprobamate is a central nervous system depressant and a major metabolite of carisoprodol. Most patients are prescribed these medications for muscular pain management or anxiety, but abuse may develop due to the sedative-hypnotic effects these compounds produce.

In San Francisco, blood evidence is screened for ethanol and related compounds by Headspace/Gas Chromatography equipped with Flame Ionization Detection (HS/GC/FID). Blood is further screened by enzyme-linked immunosorbent assay for barbiturates, cannabinoids, cocaine, methadone, methamphetamine, opiates, and phencyclidine and by Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode for over 100 drugs and metabolites. Following a positive screening result for carisoprodol and/or meprobamate by GC/MS, a fresh aliquot of blood is extracted by liquid-liquid extraction and reanalyzed by GC/MS using quantitative calibrators freshly prepared in drug-free swine blood.

For the purposes of the present study, the in-house computerized database was interrogated, and 21 driving cases in which the laboratory had reported carisoprodol and/or meprobamate in blood specimens were identified during the three-year period of interest. Police reports were reviewed and information regarding the date/time of driving, the observed driving, and field sobriety test performance was collected. The 21 drivers with reportable carisoprodol/meprobamate in their blood comprised of 6 females and 15 males. The mean age of all drivers was 32 (range: 19 – 50). The mean age in females was 38 (range: 25 – 50) and 28 (range: 19 – 44) in males. Police reports indicated that drivers freely admitted to taking carisoprodol/meprobamate, did not exhibit breath odor of alcoholic beverage, displayed glossy, watery eyes, and slurred speech. Their demeanors were described as calm and relaxed. During the horizontal gaze nystagmus, clues observed included inability to follow the object, inability to keep head still and track only with eyes, lack of convergence, and lack of reaction to light. During the Romberg test, clues observed included eyelid fluttering, opening of the eyes during the test, and estimation of 38 – 95 sec for 30 sec. The clues observed during the "one leg stand test" included poor balance, inability to keep the foot off the ground, and raising arms up to six inches to maintain balance. Clues observed during the "finger count test" included touching the finger pads instead of the tips and miscounting the steps and the number of steps performed.

Carisoprodol was reported in all 21 driving cases while meprobamate was reported in 20 of the 21 cases. The mean carisoprodol and meprobamate concentrations and associated ranges in all 21 drivers were 11mg/L (0.8 – 26mg/L) and 20.4mg/L (3.2 – 38mg/L), respectively. Carisoprodol and/or meprobamate were the only drugs detected in one-third of the cases included in this study (7 of 21 cases). In these seven cases, the mean carisoprodol and meprobamate concentrations were 12.3mg/L (7.3 – 16mg/L) and 30.4mg/L (19 – 36mg/L), respectively. In the remaining 14 cases where carisoprodol/meprobamate were not the sole compounds detected, drivers' blood specimens were found to contain on average two more psychoactive compounds including benzodiazepines (n=3), cannabinoids (n=3), oxycodone (n=3), ethanol (n=2), methadone (n=2), hydrocodone (n=2), cocaine/benzoylecgonine (n=2), methamphetamine (n=1), MDMA (n=1), citalopram (n=1), and tramadol (n=1).

Carisoprodol/meprobamate occurrence in driving under the influence cases in the City and County of San Francisco is a significant and on-going challenge. Often these drugs are present with other psychoactive compounds in drivers, making it difficult to assign specific signs and symptoms to them. Carisoprodol/meprobamate were the only drugs found in the blood of drivers in the minority of these 21 cases whereas in most of the cases, they were present together with several other compounds, primarily benzodiazepines, cannabinoids, and oxycodone.

Carisoprodol, Meprobamate, DUID

 ** Presenting Author*

K42 Cannabinoids in 113 Driving Under the Influence of Drugs (DUID) Forensic Toxicology Cases

Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103*

After attending this presentation, attendees will understand the ranges of concentrations at which cannabinoids are often detected in DUID cases.

This presentation will impact the forensic science community by providing valuable information on cannabinoid incidence among drivers and by offering blood reference concentrations of common cannabinoids in DUID case investigations.

The Forensic Laboratory Division of the Office of the Chief Medical Examiner performs DUID and other human performance forensic toxicology cases investigations on behalf of 14 law enforcement agencies routinely operating within the City and County of San Francisco. Specifically for cannabinoids, commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits by Venture Labs, Inc. are employed to screen blood and urine. The ELISA cannabinoid cutoffs used for blood and urine are 5 ng/mL and 50ng/mL, respectively. Following a positive ELISA, confirmation and/or quantitation takes place on a new specimen aliquot by Gas Chromatography/Mass Spectrometry (GC/MS) with a limit of quantitation of 1ng/mL for Δ^9-tetrahydrocannabinol (THC) and 5ng/mL for 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) for both blood and urine. The deuterated compounds THC-d_3 and THCCOOH-d_3 are used as internal standards. For THC-d_3, the target (underlined) and qualifier ions are 389 and 374. For THCCOOH-d_3, the target (underlined) and two qualifier ions are 374, 476, and 491. For THC, the target (underlined) and qualifier ions are 386 and 371. For THC-COOH, the target (underlined) and two qualifier ions are 371, 473, and 488. Finally, for THC-OH, the target (underlined) and two qualifier ions are 371, 459, and 474.

Between July 1, 2010, and June 30, 2011, the Division performed toxicologic evaluations in 919 DUID cases including the cannabinoid protocol described above. Cannabinoids were consequently reported in 113 of the 919 cases (12.3%). Five of these cases involved urinalysis and 108 involved blood analysis.

Drivers averaged 29.7 years of age (range: 14 – 68 years) and were predominantly male (n=100; 88%) which represents a higher proportion of male drivers as compared to the overall sex distribution of drivers whose toxicology was performed by this Division during the same time period (750 males, 82%; 169 females, 18%).

Among all 108 blood DUID cases, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 5 (1 – 33), 9 (5 – 14) and 52 (5 – 320), respectively.

In 64 of the 108 cases involving blood evidence, ethanol was also found at a mean concentration of 0.13% (w/v) which ranged from 0.01 – 0.38% (w/v). In four of these 64 ethanol-cannabis cases, additional drugs were also detected (diazepam/nordiazepam in two cases, alprazolam in one case, methadone in one case, and MDMA/MDA in one case). In the 64 cases where ethanol was reported in addition to cannabinoids, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 4 (1 – 23), 9 (8 – 12), and 44 (5 – 150), respectively.

Cannabinoids were reported in combination with drugs other than ethanol in 15 cases. In those 15 cases, drugs found in addition to cannabinoids were codeine (four cases), cocaine/benzoylecgonine (three cases), methamphetamine/amphetamine (three cases), MDMA (three cases), oxycodone (two cases), alprazolam (two cases), methadone (one case), and hydrocodone (one case). In these 15 cases where cannabinoids were found in combination with drugs other than alcohol, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 7 (1 – 33), 8 (7 – 10), and 65 (13 – 290), respectively.

Cannabinoids were the only compounds reported in 29 of the 108 blood DUID cases. In these 29 cases, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 7 (1 – 26), 8 (5 – 14), and 62 (6 – 320), respectively.

In the five cases involving urinalysis, THC-COOH was the only cannabinoid confirmed present. Only in one of the five urinalysis cases, cannabinoids were the only compounds reported. Cocaine/benzoylecgonine, cocaethylene, and codeine were each confirmed present in two urinalysis cases, whereas morphine, phencyclidine, carisoprodol, meprobamate, promethazine, norpromethazine, and levamisole were each confirmed present in one urinalysis case. Per professional guidelines, drug concentrations are not measured/reported in urine specimens.

This study offers a significant insight into the blood cannabinoid concentrations of drivers involved in DUID investigations in San Francisco. The reported mean concentrations suggest that drivers who concurrently consume ethanol with cannabis have on average lower THC blood concentrations than drivers who use cannabis by itself or with drugs other than ethanol and one may infer these drivers may be changing their cannabis use patterns (i.e., consuming lower cannabis doses and/or extending the waiting times before drinking) when they combine cannabis with alcohol. This type of epidemiological data provides reference concentrations for forensic toxicologists, law enforcement agents, and attorneys who are required to evaluate cannabinoid concentrations in human performance toxicologic specimens when involved in DUID investigations.

 ** Presenting Author*

Cannabinoids, Toxicology, DUID

 ** Presenting Author*

K43 Toxicology Result of Drivers of Fatal Motor Vehicle Accidents in Harris County, Texas, in 2011

Fessessework G. Guale, DVM, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054; and Ashraf Mozayani, PhD, PharmD, Texas Southern Univ, 3100 Cleburne Ave, Houston, TX 77004*

After attending this presentation, attendees will have relevant information about the involvement of illicit, prescription, and over-the-counter drugs in causing the majority of fatal traffic accidents. Attendees will learn the importance of extensive standardized testing of biological specimens for different classes of drugs in addition to alcohol.

This presentation will impact the forensic science community by raising awareness on the prevalence of fatal motor vehicle accidents caused by drugs alone with no alcohol involved. The involvement of drugs in fatal traffic accidents other than alcohol have not been adequately explored, partly due to lack of information in police training and most of the crime laboratories are neither mandated nor equipped to perform confirmation and quantitation of illicit, prescription, and over-the-counter drugs in biological specimens, especially blood. The other reason is the high cost of drug confirmation in biological specimens and the possibility of obtaining convictions based on only blood alcohol levels.

Driving under the influence of alcohol and drugs has been the main cause of fatal and non-fatal accidents for drivers and other occupants in the car, as well as pedestrians. In 2011, the medical examiner of the Harris County Institute of Forensic Sciences performed autopsies of 214 victims of fatal car crashes. All drivers of fatal motor vehicle accident cases were subject to alcohol screening and confirmation, nine-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) screens for drug of abuse, standard basic drug screen for prescription and over-the-counter drugs using Gas Chromatography/Mass Spectrometry (GC/MS), screens for 11 synthetic marijuana (Spice/k2) drugs, 8 methylcathinones (bath salt) drugs, and three hypnotic Z-drugs using Time-Of-Flight/Liquid Chromatography/Mass Spectrometry (TOF/LC/MS). All identified drugs by screening methods were subject to confirmation and quantitation by GC/MS, GC/MS/MS, and LC/MS/MS instruments. Out of 214 victims, 134 (63%) had ethanol and other drugs in their system. Out of 134 cases, 86 cases were positive for ethanol with quantitation result greater than 0.08gm/dl in 77 (89%) of cases and less than 0.08gm/dl in 9 (10.4 %) of cases. Out of 134 cases, 46 (34%) were positive for illicit, prescription, and over-the-counter drugs with no ethanol. Next to ethanol, the most common drug identified was marijuana in 30 (22%) of the cases with and without ethanol present. Ten (17%) of the cases had alprazolam, 9 (6.7%) cases had cocaine, 7 (8.9%) cases had hydrocodone, and 5 (3.7%) cases had PCP. The prevalence of alcohol and drugs among the deceased drivers' cases indicate alcohol and marijuana being the most common findings followed by benzodiazepines, opiates, cocaine, PCP, muscle relaxants, and other prescription drugs. Out of 134 drug-positive cases, 100 (74%) are male and 26% are female drivers, 63 (47%) are White, 48 (35%) are Hispanic, 27(20%) are Black, 3 (2%) are Asian and 2 (1.4%) are unknown race. The most common age group is in the range of 21 – 30 (35%), followed by 31 – 40 (20%), 41 – 50 (15%), 51 – 60 (17%), >60 (8.2%), <21 (11%). White male drivers, 21 – 30 years of age, are the most identified victims of fatal alcohol and drug related accidents.

The importance of screening and confirmation of illicit, prescription, and over-the-counter drugs in the biological specimen of motor vehicle accident victims is discussed.

Drugs, Driver, Fatality

 ** Presenting Author*

K44 Using Pharmacology to Screen Your DWI-Drug Cases

David M. Benjamin, PhD, 77 Florence St, Ste 107N, Chestnut Hill, MA 02467-1918*

After attending this presentation, attendees will be able to describe the pharmacologic criteria necessary for a prosecutor to meet the burden of proof to establish DWI-Drugs and apply these criteria to the findings of the presence of a drug in the urine.

This presentation will impact the forensic science community by presenting pharmacologic criteria which will help prosecutors select meritorious cases and defer going forward on cases when the pharmacology clearly will not support guilt beyond a reasonable doubt. An awareness of these criteria should minimize the prosecution of unwinnable cases, save valuable court time, improve the quality of justice in the courts, and protect innocent citizens from unnecessary mental anguish and monetary expense. A prototypical case will be discussed.

In order to successfully prosecute a DWI-Drugs case, the State must show that the defendant driver was impaired, and that the impairment was proximately caused by a previously ingested drug. The drug could be an illicit drug, a prescription drug, an over-the-counter drug, or an inhalant of some type. An admission by the driver that he/she took a drug earlier, or the positive results of a urine drug test, only indicate prior exposure, but do not demonstrate that the previously ingested drug proximately caused impairment at the time of the police stop. Evidence of impairment is best established by observations of erratic behavior by fact witnesses, and testing of balance, coordination, and cognitive function by professionals qualified to administer such tests.

Example of an Unwinnable DWI-Drug Case: In a Commonwealth of Massachusetts case (Com), a young woman, E.D., was stopped for crossing a road line. Her urine screen was positive for a small amount of butalbital, a barbiturate found in common prescription anti-migraine medications.

Many drugs remain in the body long after the time interval of their pharmacologic activity has elapsed (biosphere). Thus, determining the presence of a drug in a biological fluid is not a sufficient basis to opine that an individual is impaired. Instead, one must determine the biosphere of that drug, that is, the duration of time the drug exerts its pharmacologic effect on the individual, rather than its residence time in the body. Moreover, only certain drugs show a good correlation between the magnitude of their blood level and the extent of the effect they produce on the individual. Other drugs, called hit-and-run drugs, produce an effect long after they have left the body and are no longer detectable in blood or urine. A good example of such a drug would be the glucocorticoid, prednisone, which produces major changes in the hypothalamic-pituitary-adrenal axis, which may persist for weeks or months.

The half-life of butalbital is 1.5 – 3.5 days. Based on generally accepted pharmacokinetic principles, it takes 6 – 10 half-lives to rid the body of a drug. This means that E.D. could have taken the drug more than a month earlier and still had a positive urine test on the day she was stopped, even though the pharmacologic effects on migraine relief and possible impairing effects last only a few hours, and tolerance to barbiturates is known to occur after repeated use.

The COM planned to have a Drug Recognition Expert (DRE) testify at trial that the defendant had been impaired, an opinion that could not be proven beyond a reasonable doubt, and one to which the DRE was not percipient, as she had never met or assessed the defendant. A DRE is basically a fact witness, not an expert, and according to *U.S. vs. Horn* (Motion Hearing, 2001) testifies under FRE 701 not FRE 702, and cannot offer opinion testimony on scientific or technical issues. However, on the day of the *Daubert* hearing to strike the DRE, the COM reconsidered its position, and agreed to let the defendant off with probation.

In this case, a simple pharmacokinetic analysis would have indicated the very low likelihood that the prosecution could have proved its case beyond a reasonable doubt, or that a jury would have convicted. The defendant was subjected to unnecessary anxiety and considerable expense, without an adequate basis for prosecution of this case.

Pharmacokinetics, Lay Witnesses, Expert Witnesses

 ** Presenting Author*

K45 Cannabinoids in Exhaled Breath Following Controlled Administration of Cannabis

Sarah K. Himes, BS, National Institute on Drug Abuse, Biomedical Research Center (BRC), 251 Bayview Blvd, Ste 200, Rm 05A721, Baltimore, MD 21224; Karl B. Scheidweiler, PhD, 251 Bayview Blvd, Ste 200, Rm 05A721, Baltimore, MD 21224; Olof Beck, PhD, Karolinska Universitetssjukhuset Huddinge, 141 86 Stockholm, SWEDEN; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A721, Baltimore, MD 21224*

The goal of this presentation is to offer a greater understanding of the applicability and relevance of measuring cannabinoids in breath following controlled smoked cannabis. The hypothesis is that the detection window of cannabinoids in breath will be several hours. This would be an appropriate timeframe for detecting driving under the influence of drugs, as this is similar to the window of acute drug impairment.

This presentation will impact the forensic science community by supporting the application of cannabinoid breath testing, particularly in the drugged driving field.

Methods: Breath specimens were collected using SensAbues (Huddinge) devices prior to and following *ad libitum* smoking of a single 6.8% Δ^9-tetrahydrocannabinol (THC) cigarette for 10 min in occasional and chronic cannabis users. This study was Institutional Review Board- (IRB) approved and written informed consent was obtained from participants. Breath specimens were collected during a 3 min period at -18, -1, 0.5, 1, 2, 3, 4, 5, 6, 8, 10.5, 13.5, and 21 hr post-smoking. Sample preparation involved a 20 min methanolic extraction, followed by solid phase extraction on polymeric SSTHC columns (UCT). THC, 11-nor-9-carboxy-THC (THCCOOH), and cannabinol (CBN) were quantified by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) similar to a method proposed by Beck *et al*. with minor modifications.[1] Limits of Quantification (LOQs) were 50pg/pad for THC and CBN and 100pg/pad for THCCOOH. Linearity extended to 10,000pg/pad for all analytes. Extraction efficiencies for THC, THCCOOH and CBN were 33.8 34.6%, 58.1-63.0%, and 67.1-73.5% across the linear range. Matrix effects ranged from -34.6 to 12.3%. Extraction efficiencies and matrix effects were similar for matched deuterated internal standards. To date, breath specimens from one occasional and five chronic smokers were tested.

Results: Following controlled administration of THC, breath pads were positive only for THC; CBN and THCCOOH were not detected in any specimen at the method's LOQs. Breath pads were positive only at the 0.5, 1, and 2 hr post-smoking collections. Following cannabis smoking, dry mouth frequently occurred, making the collection of oral fluid difficult. As oral fluid collections occurred just prior to breath collection, breath collection times were delayed early in the time line due to prolonged oral fluid collection times. Mean (range) times for breath collection were 0.9 (0.78-0.98), 1.4 (1.30-1.45), and 2.4 hr (2.25-2.58) post-smoking. All but one participant had detectable THC in breath at 0.9 hr with a median (range, n) THC breath concentration of 147.0pg/pad (117-409, n=5). At 1.4 hr post-smoking, all but one participant had THC-positive breath with a median concentration of 122.2pg/pad (71.4-209, n=5). Only three participants still had detectable THC in breath at 2.4 hr post-smoking with a median concentration of 67.6pg/pad (54.0-86.3, n=3). For two participants, only one breath collection was positive for THC: 109pg/pad at 1.37 hr and 118pg/pad at 0.9 hr. Participants with multiple positive specimens (n=4) showed decreasing THC breath concentrations over time. In all participants, once breath specimens were negative for THC, they remained negative for the duration of the study.

Conclusions: The cannabinoid detection window in breath was short, ranging from 0.9 – 2.4 hr after cannabis smoking. Only parent THC was present; CBN and THCCOOH were not detected. Breath is an alternative matrix to oral fluid for a short cannabis detection window. Future research should determine if THC is present in breath during sustained cannabis abstinence in chronic daily cannabis smokers. During prolonged abstinence after chronic daily cannabis smoking, large THC body stores were slowly eliminated in the blood, plasma, urine, oral fluid, and sweat. The low THC concentrations in breath after cannabis smoking suggest that prolonged excretion will not occur. These data support the suitability of cannabinoid breath testing in the field of forensic science, particularly in the drugged driving field.

Reference:

1. Beck, O, Sandqvist, S, Dubbelboer, I and Franck, J. (2011) "Detection of Δ9-Tetrahydrocannabinol in Exhaled Breath Collected from Cannabis Users." J Anal Tox (35): 541-544.

Breath, Cannabinoids, THC

 ** Presenting Author*

K46 Determination of Synthetic Cannabinoids in Whole Blood From Recreational Users

Robert Kronstrand, PhD, and Markus Roman, BSc, Nat'l Board of Forensic Med, Dept of Toxicology, Artillerigatan 12, Linkoping, SWEDEN*

After attending this presentation, attendees will understand how synthetic cannabinoids may be analyzed and will have an insight in the common findings in recreational users as well as the challenges these compounds present to the forensic toxicologist. This paper presents a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for synthetic cannabinoids in whole blood and toxicological findings from recreational users.

This presentation will impact the forensic science community by adding findings for substances that have paucity in data.

Analysis was performed on a Waters ACQUITY ultra performance LC connected to an API 4000 triple-quadrupole instrument equipped with an electrospray interface. The following compounds were included in the method: JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-073-methyl, JWH-081, JWH-098, JWH-122, JWH-147, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM-2201, AM-1241, RCS-4, RCS-8, and WIN 55,212-2. JWH-018-d_{11} was used as internal standard. The analytes were chosen because they either were scheduled by the Swedish government or because they were reported as possible drugs of abuse.

To 1g whole blood was added 0.5mL TRIS-buffer (0.5M, pH=8.5) and 3mL of tert-butylmethylether:chlorobutane (50:50). The organic phase was transferred to new tubes, evaporated to dryness, and reconstituted in 100µL of 10mM ammonium formate and acetonitrile (50:50). 5µL was injected into the LC/MS/MS. Chromatography was performed using an ACQUITY high-strength silica T3 column (1.8µm, 50 x 2.1mm, Waters) and operated at 0.6mL/min with a total run time of 6 minutes. Mobile phase A consisted of 0.05% formic acid in 10mM ammonium formate and phase B was 0.05% formic acid in acetonitrile. The chromatographic system was run in a linear gradient from 35% to 70% phase B.

Method validation included selectivity and matrix effect studies, investigations of calibration models, accuracy, within and between day precision, and dilution integrity. The method was applied to 1,609 authentic cases where the police had requested synthetic cannabinoids.

The method validation experiments showed overall good results for the 23 analytes. Matrix effects were seen especially for the late eluting compounds and an interference for the transitions of RCS-8 appeared in most chromatograms close to the retention time of RCS-8. All analytes were best fitted to quadratic calibration curves between 0.05ng/g to 5.0ng/g.

The overall positive rate was approximately 30% with AM-2201 as the most common finding. The positive findings changed over time, sometimes so that substances that were scheduled decreased to be replaced by new unscheduled analogs. Eleven of the analytes were found in one or more cases.

Concentrations were typically in the subnanogram range, but some cases had very high concentrations (see table). It has been reported that synthetic cannabinoids, in comparison to cannabis, seem to be more dangerous and potent, causing several unwanted symptoms in the users. In the material, case histories were not received in more than a few cases where the subjects had suffered from severe side effects and been brought to hospital. These subjects presented with unconsciousness, vomiting, incontinence, and hallucinations and relatively high concentrations of JWH-018, JWH-203, or JWH-210, sometimes in combination with another synthetic cannabinoid. This study concludes synthetic cannabinoids appear in very low concentrations and the changing panorama of substances requires a flexible approach to the analytical methodology.

Cannabinoids, LC/MS/MS, Recreational Users

 ** Presenting Author*

K47 Driving Under the Influence of Alprazolam

H. Chip Walls, BS, Forensic Toxicology Lab, 1600 NW 10th Ave #7025A, Miami, FL 33136-1015; and Nicholas B. Tiscione, MS, Ilene K. Alford, MS, Xiaoqin Shan, PhD, and Dustin Yeatman, MS, Palm Beach County SO, 3228 Gun Club Rd, West Palm Beach, FL 33406; and Brianna Peterson, PhD, 2203 Airport Way, S, Seattle, WA 98134*

After attending this presentation, attendees will have a better understanding of the prevalence of alprazolam in DUID cases and the lack of correlation between blood alprazolam concentrations and impairment.

This presentation will impact the forensic science community by adding to the body of knowledge regarding alprazolam concentrations found in Driving Under the Influence of Drugs (DUID) cases, observed impairment, and comparison to the indicators for central nervous system depressants in the Drug Recognition Expert (DRE) matrix.

Alprazolam, a high-potency benzodiazepine, is FDA approved for the treatment of anxiety and panic disorder and is one of the most prescribed medications and abused drugs worldwide. Sedation and impairment of cognitive function and psychomotor performance are some of the main problems associated with the use of benzodiazepines as anxiolytics. Patients using alprazolam commonly report adverse events, such as drowsiness, dizziness, and reduced alertness, especially in the first month of use. Alprazolam is commonly used with alcohol and other recreational drugs, presumably to achieve increased intoxication and manage undesirable drug withdrawal symptoms such as the downside or dysphoric phase of stimulant use and to alleviate the panic and paranoia caused from using high potency cannabis. The pharmacology of alprazolam will be reviewed as an attempt to understand why Alprazolam is such a popularly abused drug in suspected intoxicated driver cases across the United States.

Between 2007 and 2012, 28% of all blood DUI drug cases submitted to the Palm Beach County Sheriff's Office Crime Laboratory contained alprazolam, making this the most commonly detected drug. Of the 205 cases analyzed during this period containing alprazolam, only nine contained alprazolam alone (4.4%). The nine subjects were composed of six males and three females with a mean age of 45 years (range 17 – 72). Seven out of nine involved traffic crashes. Alprazolam concentrations ranged from 22 to 437ng/mL.

During the first six months of 2012, 13% of all DRE cases submitted to the Washington State Patrol Toxicology Laboratory contained alprazolam. Of the 64 DRE cases analyzed during this period containing alprazolam, only seven contained alprazolam alone (10.3%). The seven subjects were composed of four males and three females with a mean age of 38 years (range 24 – 57). Alprazolam concentrations ranged from 10 to 210ng/mL.

Summary data from all sixteen alprazolam-only cases followed by detailed information for four cases from Palm Beach County and seven Washington State cases will be presented. Detailed information will include testing protocol, analytical results, and case synopsis including observed impairment and clinical indicators of drug use. The indicators for central nervous system depressants in the DRE matrix will be compared and contrasted with these case investigations.

Alprazolam, DUID, DRE

 ** Presenting Author*

K48 Prevalence of Tetrahydrocannabinol in Oral Fluid Collected From Drivers in California

Christine Moore, PhD, DSc, and Cynthia Coulter, BS, Immunalysis Corporation, 829 Towne Center Dr, Pomona, CA 91767; and Mark Johnson, PhD, John Lacey, MPH, and Tara Kelley-Baker, PhD, Pacific Institute for Research and Evaluation, 11720 Beltsville Dr, Calverton, MD 20705*

After attending this presentation, attendees will be aware of the prevalence of marijuana in the oral fluid of California drivers. Attendees will appreciate the utility of oral fluid in traffic safety situations.

This presentation will impact the forensic science community by showing that oral fluid is a viable alternative to blood for the analysis of drugs in specimens taken from drivers.

Recent roadside surveys conducted in the United States have determined that cannabis is the most prevalent drug detected in drivers randomly stopped and voluntarily tested. The 2007 National Roadside Survey reported that 7.7% of nighttime weekend drivers tested positively for tetrahydrocannabinol (THC) in their oral fluid, indicating recent ingestion of cannabis.

This presentation will focus on data collected from drivers in various locations in California during 2010 and again in 2012.

In 2010, researchers attempted to recruit 1,784 drivers who were stopped at random during nighttime hours on Friday and Saturday evenings. Due to age or type of vehicle, 282 were not selected. Of the 1,502 eligible drivers, 297 either refused to be part of the survey, or only completed part of the process. In total, oral fluid was collected voluntarily from 900 drivers. Subjects were predominantly male (63.2%), White (60.5%), and had a median age of 29. A total of 14.4% of drivers tested positively for illegal drugs with 8.5% testing positively for THC. Only 1.3% of the drivers were positive for both THC and alcohol, a combination known to significantly increase the odds of traffic accidents. Compared to the 2007 National Survey, the percentage of marijuana positives in California drivers had increased overall (from 4.9% to 7.8%) and in three of the four comparable jurisdictions; only one showed a lower percentage of positive drivers in 2010. In 2010, the prevalence of cannabis varied throughout the state with Fresno showing the lowest prevalence of 4.3% and Eureka, Humboldt County, having the highest prevalence of drivers positive for marijuana, 18.3%. The concentration range for THC in oral fluid was 2 – 1284ng/mL; mean 199ng/mL; median 30ng/mL. Part of the impetus for the 2010 study was the potential for decriminalization of marijuana in California Proposition 19—also known as the Regulate, Control, and Tax Cannabis Act of 2010—which was on the ballot in November 2010. If it had been approved, the proposition would have legalized various marijuana-related activities in California (although not under federal law), allowing local governments to regulate these activities, permitting local governments to impose and collect marijuana-related fees and taxes, and authorizing various criminal and civil penalties. The ballot initiative was defeated 53.5% to 46.5%. However, medical marijuana is legal in California (Proposition 215, 1996) and as part of the survey, drivers were asked if they held a permit for its medical use. While only 36 drivers admitted to having permits for medical marijuana, 38.9% of them tested positively for THC, compared to 7.5% of those without permits. When controlled for driver age, race, and including jurisdiction as a random variable, drivers holding permits for medical marijuana were significantly more likely to test positively for THC than nonpermit holders.

In 2012, the California Study was repeated with data collected from nine locations including Anaheim (Orange County), Chula Vista (San Diego County), Eureka (Humboldt County), Fresno (Fresno County), Gardena (Los Angeles County), Ontario (San Bernardino County), Modesto (Stanislaus County), Redding (Shasta County), and San Rafael (Marin County). Four of the nine sites were the same as the 2010 study (Anaheim, Eureka, Fresno, and San Rafael). Researchers recruited over 1,000 drivers during weekend nighttime hours to provide oral fluid specimens voluntarily. Cannabis use throughout the state was again different, depending on geographical location.

The difference in marijuana prevalence in drivers between 2007, 2010, and 2012 will be presented. The effect of medical marijuana availability and potential decriminalization of marijuana in the state of California will be discussed.

Oral Fluid, Marijuana, Driving

 ** Presenting Author*

K49 Can Oral Fluid Cannabinoid Testing Differentiate Cannabis Smoking From Intake of Oral THC and Oromucosal Sativex® Administration?

Dayong Lee, MS, National Institute on Drug Abuse, 251 Bayview Blvd, Ste 200, Baltimore, MD 21224; Erin Karschner, BA, NIDA Intramural Research Program, Chemistry & Drug Metabolism Section, 5500 Nathan Shock D, Baltimore, Maryland 21224; Allan J. Barnes, BS, NIDA Intramural Research Program, 5500 Nathan Shock Dr, Rm 373, Baltimore, MD 21224; Garry Milman, PhD, National Institute on Drug Abuse, 251 Bayview Blvd, Ste 05A.505.16, Baltimore, MD 21224; Robert S. Goodwin, DO, PhD, NIDA Intramural Research Program, Chemistry & Drug Metabolism Section, 5500 Nathan Shock Dr, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will be informed about cannabinoid disposition in Oral Fluid (OF) after a single oral Δ^9-tetrahydrocannabinol (THC) or Sativex® oral mucosal administration. Dronabinol, synthetic THC, is approved in the U.S. for treating anorexia caused by AIDS and chemotherapy-associated nausea/vomiting. Sativex®, a cannabis oromucosal spray containing approximately 1:1 THC and cannabidiol (CBD), is in phase III trials for cancer pain in the U.S. and approved for multiple sclerosis symptoms in several European countries, Canada, and New Zealand. While several studies investigated OF cannabinoid pharmacokinetics following cannabis smoking, there is only one with multiple oral THC doses and none with Sativex®. This presentation characterizes OF cannabinoid time-course profiles, windows of detection, and cannabinoid ratios after the oral and sublingual drug delivery routes. Differences from cannabis smoking were evaluated for possible approaches to identifying relapse and compliance with cannabinoid pharmacotherapy.

This presentation will impact the forensic science community by providing the first data defining cannabinoid disposition in oral fluid after single-dose medicinal cannabinoid products. These data will improve interpretation of oral fluid cannabinoid testing and aid in formulating policy and legislation for oral fluid testing and in effectively managing patients undergoing cannabinoid pharmacotherapy.

Methods: Fourteen cannabis smokers (aged 19 – 43 yrs, 79% male, 64% African American) provided written informed consent for this double-blind, double-dummy, within-subject, Institutional Review Board-approved study. The participants resided on a closed research unit at least 10h prior to each drug administration. Five or 15mg synthetic oral THC; two (low dose, 5.4mg THC, and 5.0mg CBD) or six (high dose, 16.2mg THC, and 15.0mg CBD) actuations of Sativex®; or placebo oral THC and six placebo Sativex® actuations were administered in random order. Dosing sessions were separated by at least five days. OF specimens were collected with the Quantisal™ collection device, 0.5 hr before and 0.25, 1, 4.5, 7.5, and 10.5 hr after dosing initiation. THC, CBD, cannabinol (CBN), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) were quantified by 2D Gas Chromatography/Mass Spectrometry (GC/MS). If analyte concentrations exceeded the upper limit of linearity, participants' OF specimens were diluted with drug-free OF-Quantisal™ buffer mixture. Limits of quantification were 0.5ng/mL for THC, CBD, and 11-OH-THC, 1ng/mL for CBN, and 7.5pg/mL for THCCOOH.

Results: After oral THC, OF THC decreased over time from baseline concentrations ≤20.5ng/mL; concentrations were not significantly different from those after placebo, reflecting residual THC excretion from previously self-administered smoked cannabis. CBD and CBN were only detected in three specimens with concentrations ≤1.1ng/mL. After Sativex®, THC, CBD and CBN increased greatly with peak concentrations of 266 – 11,424, 196 – 12,120, and 9.5 – 560ng/mL (low dose, respectively); 1,323 – 18,216, 1,552 – 18636, and 74 – 22,32ng/mL (high dose, respectively) occurring at 0.25 – 1 hr, except one CBD at 4.5 hr. After low- and high-dose Sativex®, all specimens were positive for THC and CBD until 10.5 hr post-dose with concentrations 1.0 – 92.0 and 0.5 – 131ng/mL, respectively. After low- and high-dose Sativex®, 43% and 79% of specimens, respectively, were CBN-positive for 10.5 hr with concentrations ≤6.9ng/mL. Median (range) CBD/THC and CBN/THC ratios were 0.82 – 1.34 (0.27-2.26) and 0.04 – 0.06 (0.01-0.52), respectively, over 0.25 – 10.5 hr. In comparison, median (range) CBD/THC and CBN/THC ratios after smoking a single 6.8% THC cigarette were 0.04 – 0.05 (0.03-0.09) and 0.07 – 0.08 (0.04 –0.15), respectively, within 0.25 – 6 hr post dose. OF THCCOOH concentration changes over time were less evident and significantly masked by baseline concentrations in all dosing sessions. THCCOOH/THC ratios were <4pg/ng for 4.5 and 1 hr post Sativex® and smoked cannabis, respectively, while ratios were never below 4pg/ng after oral THC and placebo. THCCOOH/THC ratios increased over time in each dosing session.

Conclusions: Oral THC and Sativex™ administered in low and high dosages produced OF cannabinoid disposition different from those after smoked cannabis; THC, CBD, and CBN were rarely detected after oral THC while Sativex® generated high CBD/THC ratios. Low THCCOOH/THC ratios suggest recent Sativex® and smoked cannabis exposure. Study results indicate that relapse to smoked cannabis during oral THC pharmacotherapy for cannabis dependence should be evident with OF cannabinoid monitoring. In contrast, compliance with Sativex® pharmacotherapy should be clearly apparent by the high OF CBD/THC ratio as compared to that following cannabis smoking; however, additional research is needed to determine if relapse to cannabis smoking can be identified during Sativex® pharmacotherapy, as the high OF CBD/THC ratio after Sativex® may not be altered

 ** Presenting Author*

sufficiently to identify single smoked cannabis episodes. Interpretation of OF cannabinoid tests will be improved by these data, the first defining OF cannabinoid disposition after single-dose medicinal cannabinoid products. These data also are valuable for formulating policy and legislation for OF testing, and for effectively managing patients undergoing cannabinoid pharmacotherapy.

Oral Fluid, Cannabinoids, Cannabis

 ** Presenting Author*

K50 Current Research Initiatives in Toxicology at the National Institute on Drug Abuse

Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A721, Baltimore, MD 21224; Ariane Wohlfarth, PhD, and Rebecca L. Hartman, BA, National Institute on Drug Abuse, 251 Bayview Blvd, Baltimore, MD 21224; and Nathalie A. Desrosiers, MSc, 251 Bayview Blvd, Ste 200, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will be able to describe new research from Chemistry and Drug Metabolism (CDM), detailing National Institute on Drug Abuse (NIDA) findings on urinary cannabinoid excretion, oral fluid cannabinoid stability, Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) confirmation of urinary synthetic cannabinoids, and performance impairment and blood THC concentrations in driving cases.

This presentation will impact the forensic science community by revealing CDM investigations of illicit drug agonists, antagonists, and drug dependence treatment pharmacotherapies. Controlled drug administration studies in drug users were conducted under Institutional Review Board-approved protocols and Investigational New Drug applications from the Food and Drug Administration.

CDM investigates the pharmacodynamics and pharmacokinetics of illicit drug agonists and drug dependence pharmacotherapies. Phase I controlled drug administration studies are conducted in drug users under Institutional Review Board-approved protocols and Investigational New Drug applications from the U.S. FDA. Recently, this study focused on mechanisms of action of cannabinoid agonists, development of evidence-based drug policy, and legislation for oral fluid testing and emerging designer drugs. In this presentation, new research findings are shared on urinary cannabinoid excretion, oral fluid cannabinoid stability, LC/MS/MS confirmation of urinary synthetic cannabinoids, and performance impairment and blood THC concentrations in driving cases.

Twelve chronic frequent and nine occasional cannabis users smoked one 6.8% Δ^9-tetrahydrocannabinol (THC) cigarette. Cognitive, subjective, psychomotor and physiological responses, and urinary cannabinoid pharmacokinetics were characterized. THC, cannabidiol (CBD), cannabinol (CBN), 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), THC-glucuronide, and THCCOOH-glucuronide concentrations were simultaneously quantified by LC/MS/MS and normalized to urine creatinine. THCCOOH (frequent N=12; occasional N=6), THC-glucuronide (frequent N=12; occasional N=9), and THCCOOH-glucuronide (frequent N=12; occasional N=9) were identified; THC, 11-OH-THC, CBD, and CBN were not detected. Highest concentrations (ng/mg creatinine) in frequent and occasional cannabis smokers, respectively, were: THC-glucuronide 3.5-60.7 and 3.0-35.1; THCCOOH 2.7-17.2 and 0-7.2; and THCCOOH-glucuronide 146-548 and 20.8-298. Concentration-time curves for the excretion of urinary cannabinoids and the presence of potential markers for recent cannabis smoking will be presented.

Analyte stability is critical for interpreting drug concentrations, although there are few data on cannabinoid stability in oral fluid, an important new drug testing matrix. Cannabinoid stability in authentic oral fluid collected with the StatSure® and Oral-Eze® collection devices after controlled smoking of one 6.8% THC cigarette was evaluated. Stability pools were prepared for each participant (n=16) by combining oral fluid collected in the first 13.5 hr. Pools were aliquoted into polypropylene cryotubes and stored at room temperature (RT), 4°C, or -20°C. Baseline specimens were quantified within 24 hr, and the remaining aliquots analyzed after one week at RT and 4°C, and four weeks at 4°C and -20°C. Specimens were considered stable if concentrations were within ± 20% of baseline. Specimens collected with the Oral-Eze® and StatSure® devices were stable for THC and THCCOOH at 4°C for one week and one month, while after longer frozen storage a small number of specimen concentrations increased or decreased more than ± 20%. Mean THC and THCCOOH oral fluid concentrations were 93.0 – 96.7% of target after refrigeration for one or four weeks, and 89 – 117% (THC) and 81 – 110% (THCCOOH) for one month frozen. Elution and stabilizing buffers in oral fluid collection devices help maintain cannabinoid concentrations in oral fluid, as well as improving cannabinoid recovery from the collection pad.

Synthetic cannabinoids are an important new designer drug class. Assays are needed to identify these drugs in human urine. An LC/MS/MS method was developed for the qualitative confirmation of ten synthetic cannabinoids (JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM 2201, and RCS-4) and their hydroxyalkyl, hydroxyindole, and carboxy metabolites in human urine. Specimen preparation includes hydrolysis and protein precipitation, followed by monitoring of a single MRM transition in a survey scan that triggers an enhanced product ion (EPI) scan at three different collision energies. This information-dependent acquisition experiment is conducted on an ABSciex 5500 QTrap. Qualitative results from several hundred authentic urine specimens will show prevalence of parent and metabolites and metabolite patterns.

Finally, this latest investigation on cannabis effects on driving is presented. In collaboration with other toxicologists, driving under the influence of drugs (DUID) cannabis cases were compiled and analyzed and police reports on apprehended drivers under the influence of cannabis and blood THC concentrations were evaluated. Representative individual case reports with varying THC concentrations are presented, as well as aggregate/summary statistics. The case reports focus on cannabis-only cases, to avoid complications imposed by polypharmacy.

 ** Presenting Author*

Urine Cannabinoids, Oral Fluid Stability, Designer Drugs

 ** Presenting Author*

K51 Detection and Quantification of Antidepressants in Aqueous Matrices

Kenton J. Chodara, BS, 710 S Atherton, Apt 203, State College, PA 16801; and Dan G. Sykes, PhD, and Melissa M. Gettle, 330 Whitmore Laboratory, University Park, PA 16802*

The goal of this presentation is to delineate the process of developing a detection method capable of simultaneously identifying and quantifying antidepressant compounds in aqueous matrices using a Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS), the determination of quantitative differences between detection methods, the effect of stationary phase composition on the separation of analytes, and applications to wastewater samples pursuant to a relevant EPA method.

This presentation will impact the forensic science community by showing how the methodologies and results presented will provide widely applicable LC/MS/MS method for the detection of small molecules in a variety of aqueous matrices, including wastewater, blood, and urine. The development process can be adapted to produce a quantitative screening method for any compounds of interest suitable for LC/MS/MS identification.

Antidepressants are psychiatric medications that are taken with the intent to alleviate mood disorders. These drugs and their metabolites enter the environment as a byproduct of use, and may pose a danger to the health of humans and the environment. In the U.S., there are few regulations concerning the discharge, fate, and transport of such pharmaceuticals, many of which remain biochemically active after passing through current treatment processes. An important first step toward developing proper regulation is the creation of a selective and sensitive detection method in relevant matrices.

This presentation will discuss the methodology developed for the detection and identification of a variety of commonly used antidepressant drugs and their metabolites. High Pressure Liquid Chromatography (HPLC) on fused core silica phases is used for the separation of analytes, and a triple-quadrupole mass spectrometer is employed using both Scheduled Multiple Reaction Monitoring (s-MRM) and Information Dependent Acquisition (IDA) detection methods with Electrospray Ionization (ESI). Sample preparation is performed in accord with EPA method 1694, using solid-phase extraction with an Oasis® HLB cartridge suitable for retention of acidic, basic, and neutral compounds.

A library containing reference spectra was created using direct syringe pump infusion of standards of each antidepressant in methanol. Generalized parameters capable of ionizing and fragmenting each compound were optimized and compiled. The composite method was used to create and assess the efficacy of identifying and quantifying components of a mixture using both SMRM and IDA.

Preliminary testing was performed on a C18 column, the currently most-used column for analysis of antidepressants. The variance of s-MRM, which scans for analytes at specified expected retention times, was determined to be within acceptable limits to provide reproducible results. Information Dependent Acquisition, a method that scans using s-MRM and produces additional library matching spectra for analytes with intensities over a certain threshold, supposedly at a cost of quantitative reproducibility. The difference in quantification between the two methods was determined using labeled analogues of each compound as internal standards.

The optimized detection method was then applied to chromatographic separations using different stationary phase compositions including C8, C18, phenyl-hexyl, and amide. The goal was to determine how chemical interactions between analytes and the column influence separation and analysis. Parameters such as efficiency, reproducibility, and selectivity were considered in method optimization. Retention times, elution order, and peak shapes were compared when possible.

Antidepressant, LC/MS, Wastewater

 ** Presenting Author*

K52 ∆9-Tetrahydrocannabinol, 11-Nor-9- Carboxy-Tetrahydrocannabinol, Cannabidiol, Cannabinol, and 11- Hydroxy-Tetrahydrocannabinol in Oral Fluid Following Controlled, Smoked Cannabis in Frequent and Occasional Smokers

Sebastien Anizan, PhD, National Institute on Drug Abuse, 251 Bayview Blvd, Baltimore, MD 21224; Garry Milman, PhD, National Institute on Drug Abuse, 251 Bayview Blvd, Ste 05A.505.16, Baltimore, MD 21224; Nathalie A. Desrosiers, MSc*, NIDA Intramural Research Program, 251 Bayview Blvd, Rm 05A721, Ste 200, Baltimore, MD 21224; Allan J. Barnes, BS, NIDA Intramural Research Program, 5500 Nathan Shock Dr, Rm 373, Baltimore, MD 21224; David A. Gorelick, MD, PhD, National Institute on Drug Abuse, 5500 Nathan Shock Dr, Baltimore, MD 21224; and Marilyn A. Huestis, PhD*, Chemistry & Drug Metabolism Section, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will understand pharmacokinetic differences in Oral Fluid (OF) concentrations between chronic frequent and occasional cannabis smokers.

This presentation will impact the forensic science community by improving interpretation of oral fluid test results for different populations of cannabis smokers and may suggest recent cannabis use by selecting different analytes and cutoff concentrations.

In a previous study, additional cannabinoid analytes and cutoff concentrations were proposed to reduce the possibility of a positive cannabinoid OF test from environmental cannabis smoke contamination; however, the data only included chronic frequent cannabis smokers.[1] The objectives of the present study were to compare pharmacokinetic data from chronic frequent and occasional cannabis smokers, and to evaluate different cannabinoid analytes and cutoff concentrations to distinguish recent smoking from residual excretion in these populations.

This study consisted of healthy, 18 – 45-year-old cannabis smokers, who used cannabis at least four times per week (frequent smokers) or a maximum of two times per week (occasional smokers), provided written informed consent for this Institutional Review Board-approved study, and resided on the secure research unit for two days. OF specimens were collected with the Statsure™ device, 14 hr and 1 hr before and up to 30 hr after *ad libitum* smoking of a 6.8% ∆9-Tetrahydrocannabinol (THC) cigarette. Specimens were analyzed within 24 hr following collection. THC, 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), cannabinol (CBN), and 11-hydroxy-THC (11-OH-THC) were quantified by 2D-GC/MS. Limits of quantification (LOQ) were 0.5 ng/mL for THC, CBD, CBN, and 11-OH-THC, and 15pg/mL for THCCOOH.

Eighteen subjects (eleven chronic frequent and seven occasional cannabis smokers) provided 306 OF specimens. Fourteen hours before smoking, all chronic frequent smokers' OF tested positive for THC (range 6-396.5ng/mL) and THCCOOH (23-124pg/mL), whereas all occasional smokers' OF specimens were negative for both analytes. One hour prior to dosing, nine chronic frequent smokers' OF specimens were still positive for THC (range 0.9 to 7ng/mL), while THCCOOH was always measurable in this population (range 2.0 – 13.6pg/mL).

No significant differences ($p>0.05$) in THC concentrations were observed 2h after cannabis smoking between chronic frequent and occasional smokers. OF THC concentrations were highly elevated for 2h with medians (range) of 517.3ng/mL (113.8 – 6,508), at 0.5 hr, 238.5ng/mL (28.4 – 6,362) at 1 hr and 72.5ng/mL (7.5 – 350.5) at 2 hr for the chronic frequent smokers. Medians (range) in occasional smokers were 481.8ng/mL (84.5 – 1471.3) 0.5 hr after smoking, 82.8ng/mL (48.4 – 561.5) at 1 hr, and 69.3ng/mL (23.4 – 213.7) at 2 hr. Eight of eleven chronic frequent smokers' OF specimens were still THC-positive at 30h (0.6 – 2.2ng/mL), whereas only one of seven occasional smokers was positive (0.5ng/mL). OF THCCOOH concentrations showed large differences between chronic frequent and occasional smokers at all time points: Results showed 83% of all specimens from occasional smokers were negative (all medians equal to 0) and 95% of OF specimens from chronic frequent smokers were positive. For both groups, CBD and CBN concentrations were maximal 0.5 hr after smoking, decreasing rapidly over time. The last positive OF CBD occurred 10.5 hr after smoking in two chronic frequent smokers (0.5 – 0.6ng/mL) and at 6 hr for one occasional smoker. For CBN, four chronic frequent and one occasional smokers' OF were still positive at 13.5 hr (0.6 – 1 and 0.6ng/mL, respectively). 11-OH-THC was not present in OF except when THC concentrations were greater than 1,000ng/mL.

At a cutoff concentration of 2ng/mL THC, proposed by the Substance Abuse and Mental Health Services Administration, 72% and 71% of chronic frequent and occasional smokers were positive for 21 hr and 10.5 hr, respectively.

These findings improve interpretation of cannabinoid OF concentrations in the workplace, cannabis dependence treatment, motor vehicle accidents, and doping in sports cases.

Reference:

1. Lee D, Schwope DM, Milman G, Barnes AJ, Gorelick DA, Huestis MA. Cannabinoid disposition in oral fluid after controlled smoked cannabis. Clin Chem. 2012;58:748-56.

Oral Fluid, Tetrahydrocannabinol, Cannabinoids

 ** Presenting Author*

K53 Laboratory Based Evaluation of Commercially Available Oral Fluid Testing Devices

Amanda L. Arntson, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Ave, Willow Grove, PA 19090; and Bill Ofsa, MS, Matthew M. McMullin, MS, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to assess sensitivity and specificity, and detection thresholds of several commercially available oral fluid testing devices designed for use in the field, as well as issues with respect to their readability, robustness, and ease of use.

This presentation will impact the forensic science community by highlighting factors to be considered when selecting field-based testing devices for the presence of drugs in oral fluid.

Oral fluid is increasing in popularity as a biological matrix for drug testing in the workplace, probations and parole, and traffic law enforcement.

Three devices were selected for comparison with the Drager® DT5000 7-panel, based on their price, general availability, and advertised ease of use. The devices evaluated were the Oral Fluid 6 Drug Test (Oral Q®), Alere iScreen®, and Xalex™. Nine blind controls containing a total of 12 drugs representing the classes of amphetamines, MDMA, opiates, benzodiazepines, PCP, cocaine, THC, methadone, oxycodone, and dextromethorphan were prepared in synthetic oral fluid. An open positive (100ng/mL) and drug-free oral fluid negative control were also used.

The concentrations of the target analyte were selected so evaluations could be made below the listed cutoff concentration, near the cutoff concentration, and significantly above the advertised cutoff of the device.

Each device was evaluated in triplicate for each control group with the results being independently verified by two different individuals. The testing protocol used was specific to the device, following a protocol based on the device instructions. In devices with a sorbent sponge, the sponge was saturated with the oral fluid control mixture and subsequently tested as directed. After the verification of the results, the performance of each device was evaluated by drug class and how the device performed around its stated cutoff concentration. For each device, the sensitivity, specificity, and accuracy were assessed.

Positive results were scored as true positives if the analyte was present in the control, irrespective of its concentration. With all negative results, the concentration in the control was compared to the manufacturer's cutoff concentrations and determined if the result was a true positive or true negative relative to that cutoff.

The Drager® DT5000 was an instrumented test with an electronic analyzer generating a printed result. The remaining devices were visually read. These three had cannabinoid tests that were targeted to carboxyTHC which is known to be excreted at very low concentrations in oral fluid. The Xalex™ and Alere iScreen6 did not give a positive cannabinoid result at 100ng/mL of THC. The OralQ gave false positive results for cannabinoids in every negative control. The Drager® DT5000 did not detect the presence of THC at the positive control concentration of 7ng/mL, in spite of its published cutoff of 5ng/mL. Controls at 15ng/mL all tested positive.

The Drager® DT5000 had lower cutoffs for benzodiazepines, methamphetamine, and opiates. The OralQ® had an elevated cutoff for benzodiazepines at 50ng/mL. The Xalex™ and Alere iScreen6® devices did not include a benzodiazepine test. The Drager® DT5000 gave positive results for benzodiazepines at its advertised cutoff of 15ng/mL.

The sensitivity and specificity results for the Xalex™ and iScreen6® did not include scoring from the THC panel because the target analytes were THC metabolites, which would not be expected at the advertised cutoff in oral fluid. Absent this consideration, the Xalex™ device had sensitivity, specificity, and accuracy of 100%, and the Alere iScreen6® had 95% sensitivity, 93% sensitivity, and 94% accuracy. The OralQ® had the lowest sensitivity at 65%, specificity of 86%, and accuracy of 75%. It generated 16 false negative results relative to its advertised cutoffs across several drug classes. The Drager® DT5000 had 97% sensitivity, 100% specificity, and 98% accuracy.

Based on this initial evaluation, it was concluded that the Drager DT5000 gave the best overall performance and lacked the issue of subjectivity in reading the test strips. This laboratory-based assessment, however, indicated it had higher sensitivity for THC than advertised. Additional devices are in the process of being evaluated.

DUID, Oral Fluid, Field Test

 ** Presenting Author*

K54 Simultaneous Analysis of Opiates and Acetaminophen With Noscapine Monitoring

Jeffrey P. Walterscheid, PhD, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054; Ashraf Mozayani, PhD, PharmD, Texas Southern Univ, 3100 Cleburne Ave, Houston, TX 77004; and Michael Chen, PhD, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054*

After attending this presentation, attendees will learn about a novel liquid extraction solvent and acquisition cycle for determining opiates in forensic toxicology specimens. This extract is suitable for analysis by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) to measure various common opiates, along with acetaminophen as a co-compound and monitoring of noscapine as an alkaloid contaminant from illicit morphine preparations. The procedure is a robust and sensitive method for routinely evaluating blood, stomach, urine, and vitreous humor for evidence of opiate use and abuse. The audience will also learn about the opiate levels commonly found in postmortem death investigations, DUI suspects, and sexual assault victims. In addition, heroin deaths involving related concentrations of 6-monoacetylmorphine, codeine, and morphine will be reconciled with the presence of noscapine.

This presentation impacts the forensic science community by distinguishing a new way to analyze opiates with advantages on enhanced recovery, preparation economy, time savings, and signal stability. Since implementing this procedure over the last year, not one analytical run failed to meet acceptance criteria. Sample volumes have been reduced by half, turnaround times have been decreased, and more information is obtained without a separate extraction for acetaminophen analysis. These benefits raise services for clients in the community and help to achieve goals for laboratory accreditation.

Opiates are an important category in forensic toxicology for their prevalence in impaired drivers and overdose deaths. Another factor often overlooked is that acetaminophen is a toxicologically significant drug frequently compounded with hydrocodone tablet preparations. Although Solid Phase Extraction (SPE) purifications are a useful way of obtaining clean drug extracts for instrumental analysis, acetaminophen recovery suffers greatly. A multiple-targeted analysis of opiates consisting of morphine, hydromorphone, oxymorphone, codeine, oxycodone, 6-monoacetylmorphine, acetaminophen, and hydrocodone combined with noscapine monitoring using a modified Liquid-Liquid Extraction (LLE) has been developed. The simple LLE method utilizes a mixture of organic solvents consisting of isopropyl alcohol, isoamyl alcohol, and 1-chlorobutane for extraction to yield enhanced recovery that saves money and time.

In the LC/MS/MS acquisition method, two separate mass spectroscopy segments were utilized to improve the detection sensitivity, while short dwell times were used for acetaminophen transitions to lower sensitivity in adjusting for its relatively high concentration scale. The opiates follow a linear curve fit from 10ng/mL to 2,000ng/mL with a corresponding curve range of 1mg/L to 200mg/L for acetaminophen. The 15-minute chromatography gradient cycle allows for clean separation of all analytes, especially morphine and hydromorphone as well as codeine and hydrocodone, which are isobaric pairs that can interfere with each other in qualitative and quantitative confirmations.

This method has many benefits for the toxicology laboratory at many levels. Extraction analysts find the process to be faster and easier than SPE protocols. Instrument data processors report stronger responses with sharp peaks, linear curve fits, and quality controls in good agreement with expected outcomes. Laboratory managers realize successful sequential analytical runs decrease turnaround time, which reduces the burden on consumable budget, and provides more data using less specimen. Overall, this method has been validated as a superior process for routine forensic toxicology opiate analysis.

Opiates, LC/MS/MS, Toxicology

 ** Presenting Author*

K55 Method Development and Validation for the Analysis of Cannabinoids in Meconium Samples

Marykathryn Tynon, BS, 2901 Burlington Ave, Delanco, NJ 08075; and Marcellino Porto, MSFS, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to compare different solid-phase extraction methods for the cleanup of meconium samples prior to Three-Dimensional/Gas Chromatography/Mass Spectrometry (3D/GC/MS) analysis.

This presentation will impact the forensic science community by providing perspectives on how to assess and improve methods for extracting, isolating, and measuring cannabinoids and their metabolites from meconium.

The purpose of this project was to develop an extraction method and instrumental parameters for GC/MS and 3D/GC/MS methods for the analysis of tetrahydrocannabinol (THC), 11-OH-THC, and THC Carboxylic Acid (THCC) that meet laboratory requirements for accuracy and precision and is cost and time efficient. The project resulted in development of an improved screening for the presence of drugs in meconium samples in aid of diagnosing and detecting marijuana use by the mother during gestation.

Collectively, the components of the marijuana plant, *Cannabis sativa*, are known as cannabinoids, and have a variety of pharmacological effects in humans including, but not limited to, analgesia, appetite suppression, hypertension, euphoria, and suppression of nausea. The main active component, THC, is broken down by the liver into a variety of oxidized metabolites. The major route of metabolism is hydrolysis of THC at carbon-11 to form 11-OH-THC which is further oxidized to form 11-Nor-carboxy-THC (THCC). These three components are the major focus of most marijuana testing in human bodily fluids. Prenatal exposure of THC is thought to possibly have detrimental effects, including effects on the systems involved in emotions and maturation. Animal studies have shown that rat pups prenatally exposed to THC could have long-lasting neurological effects. Many drug court and monitoring programs require the mother to abstain from marijuana use while pregnant to avoid exposure of the developing fetus to cannabinoids, and methods are needed to monitor their compliance with those orders.

Comparisons of several variables for the isolation of cannabinoids from meconium were performed, including the comparison of two different brands of THCC-specific Solid Phase Extraction (SPE) columns, Agilent™ and Strata X™, the length of incubation while hydrolyzing the samples, the effect of homogenizing the meconium sample before extraction, the wash solvents used, and the effects of the polarity of the GC/MS columns on which the samples were run.

The significance of these variables was evaluated by performing the same extraction with only the variable in question being altered. The general extraction method includes adding 0.25g of samples to phosphate buffer pH 7.0 and adding 25µL of 12M potassium hydroxide to hydrolyze the samples. After hydrolysis, the samples were neutralized using hydrochloric acid. The neutralized samples were then treated with acetonitrile precipitation and centrifugation before pouring the supernatant onto the SPE columns. The columns were the washed, eluted, and the extract dried down before derivatization with BSTFA.

From the comparison between the two SPE columns, it was determined that the Strata X™ columns had a better recovery of THCC with the extraction method used while the Agilent™ columns had a better 11-OH-THC recovery. The percent recovery of THCC and 11-OH-THC while using the Agilent™ columns was determined to be 91% and 66.5%, respectively. The Strata X™ columns resulted in a 95% and 56% recovery for THCC and 11-OH-THC, respectively. The incubation-time evaluation led to the conclusion that while there was little change in recovery from commercially available control samples (ElSohly Labs), an authentic THC-positive meconium control sample showed significantly increased abundance of free THCC at 30 min incubation at 60°C.

Applying the optimized extraction method using deuterated internal standards for all three analytes generated calibration curves with R^2 values greater than 0.998.

The study concluded that SPE analysis of meconium samples gave cleaner extracts than liquid/liquid extraction, that hydrolysis improved recovery of the drug from the sample, and that combined with GC/MS, SPE produced calibration curves that met laboratory requirements.

Cannabinoids, Meconium, GC/MS

 ** Presenting Author*

K56 Effect of Ethanol on Succinyl Semialdehyde Dehydrogenase—Implications for Exacerbation of GHB Toxicity

Robert H. Powers, PhD, CT Dept of Public Safety, Controlled Substances/Tox Lab, 278 Colony St, Meriden, CT 06451; Robert M. Lockwood, MSFS, Univ of New Haven, Forensic Science Dept, 300 Boston Post Rd, West Haven, CT 06516; and Sophia M. Panaccione, Kings Coll Dept of Neuroscience, 133 N River St, Wilkes-Barre, PA 18711*

After attending this presentation, attendees will gain a greater understanding of the central nervous system, its endogenous inhibitory neurotransmitters and their metabolism, and the effects of ethanol on part of that metabolic process.

This presentation will impact the forensic science community by offering a mechanistic explanation for one aspect of the combined effects of ethanol/GHB co-ingestion. This knowledge will help forensic pathologists and toxicologists evaluate and interpret drug results in DFSA cases where both ethanol and GHB are determined to have been present.

Drug Facilitated Sexual Assault (DFSA) cases routinely involve the use of central nervous system (CNS) depressant agents such as benzodiazepines, barbiturates, and more recently, γ-hydroxybutyrate (GHB). A common mechanistic basis for the actions of these agents is an effect on GABA-nergic inhibitory neurotransmission. GABA (γ-amino butyric acid) is the primary inhibitory neurotransmitter in the CNS, functioning as a post-synaptic ligand-gated chloride channel agonist (GABA receptor agonist). Activation of the GABA receptor by either endogenous GABA, or by xenobiotics, results in the influx of chloride ion into the post-synaptic neuron, resulting in a hyperpolarization (inhibition) of that neuronal membrane. Removal of GABA from the synaptic space following the neurotransmission event is a function of uptake and catabolism of GABA by astrocytic cells in proximity to the synapse rather than re-uptake directly into the pre-synaptic neuron. Succinyl Semialdehyde Dehydrogenase (SSADH) is a central enzyme in the oxidative degradation of GABA and GHB, converting their common oxidative metabolite, succinyl semialdehyde (SSA), to succinate as an end product. SSA is produced directly from GABA by an enzyme-catalysed transamination (with alpha-keto glutarate, (αKG)), and from GHB by a GHB-dehydrogenase (GHBDH)-catalysed oxidation. GHB dehydrogenase is a cytosolic enzyme reducing NAD^+ as a cofactor, while SSADH and the transaminase are mitochondrial enzymes, with SSADH reducing $NADP^+$ as a cofactor.

Ethanol is commonly found in DFSA cases, either alone or in combination with other CNS depressants, including GHB, and its presence may have an impact on the interpretation of drug findings in such cases. Ethanol has been shown to exacerbate the effects of GHB; however, a mechanistic basis for that effect has not been demonstrated. It has been hypothesized that one consequence of alcohol ingestion in the body is an inhibition of SSADH by both ethanol and its oxidative metabolite, acetaldehyde, because of the structural homology between ethanol, acetaldehyde, and carbons 3 and 4 of SSA. Inhibition of SSADH would be expected to increase the effective half-life of GABA in the body, with the consequential increase in background GABA concentration, and GABA-mediated CNS depressant activity. Initial experiments with a combined enzyme system consisting of GABA-αKG transaminase/SSADH indicated that ethanol inhibited enzyme activity at a concentration equivalent to 0.4 g/dL, but did not do so appreciably at a concentration equivalent to 0.1g/dL, suggesting that any such effect of ethanol on SSADH would only be a factor in significant alcohol ingestions. Kinetic evaluation of initial reaction rates by UV spectrophotometry (monitoring generation of NADPH) indicated that ethanol affected SSADH rather than the GABA-αKG transaminase. Substrate-velocity experiments indicated that SSADH in the preparation had a Michaelis constant (K_m) for SSA of 49 μM in the absence of ethanol, and 61 μM in the presence of 0.4g/dL ethanol, as determined by Lineweaver-Burke plot. Maximal velocity (V_{max}) of the enzyme was unaffected by the inclusion of ethanol, a pattern consistent with competitive inhibition.

Based on the effect of ethanol on SSADH, it is suggested that the ingestion of alcohol in the body would, in a concentration-dependent manner, inhibit SSADH, thereby decreasing the rate of GABA- and/or GHB-derived SSA oxidation, and potentially increasing both the half-life of endogenous GABA and exogenous GHB. This effect may play a contributory role to the CNS depressant consequences of significant ethanol ingestions and combined ethanol-GHB exposures, such as could be seen in some DFSA cases.

Ethanol, GHB, GABA

 ** Presenting Author*

K57 Incomplete Recovery of Codeine in Urine Using Common Enzymatic Hydrolysis Procedures

Sumandeep Rana, MS, Tara Brunson, BS, and Wayne B. Ross, MCLS, Redwood Toxicology Laboratory, 3650 Westwind Blvd, Santa Rosa, CA 95403*

The goal of this presentation is to inform attendees about the effectiveness of various commonly used hydrolysis techniques and conditions for the hydrolysis of opiates, stressing the incomplete hydrolysis of codeine following common enzymatic hydrolysis procedures.

This presentation will impact the forensic science community by demonstrating that choosing the right combination of hydrolyzing agent and hydrolysis conditions is critical to accurate results and leads to significant improvement in recovery of opiates from urine samples.

Methods: Opiates included in this method are: morphine, hydromorphone, codeine, and hydrocodone. Deuterated analogues of all four analytes are used as the internal standards.

Extraction: Authentic urine samples were hydrolyzed using β-glucuronidase from *Escherichia coli and Helix-Pomatia* for 3 hr and 16 hr each. Samples were spiked with internal standard, centrifuged and supernatant was diluted with mobile phase before injecting on the column. In separate experiments, the amount of enzyme added was doubled to evaluate optimal concentration of the enzyme for efficient hydrolysis. In addition, one set of samples was hydrolyzed using acid hydrolysis with 0.1N HCl and the results were used as the reference (100% recovery) to evaluate recovery from different enzymatic procedures.

Analysis: Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) analysis was performed in Electronic Spray Ionization (ESI) mode by Multiple Reaction Monitoring (MRM) using a 3200 triple quadrupole mass spectrometer connected to a Shimadzu prominence HPLC system. Separation was achieved on an ultra II biphenyl 5µ column (50 X 2.1mm). Mobile phases were 0.1% formic acid and 0.2% ammonium formate in de-ionized water (A) and in acetonitrile with 2% water (B). All analytes were eluted within four minutes. Two ion transitions for each analyte; morphine (286/152, 286/128), hydromorphone (286/185, 286/157), codeine (300/152, 300/115), hydrocodone (300/199, 300/128), and one ion transition for each internal standard; morphine-D6 (292/152), hydromorphone-D3 (289/185), codeine-D6 (306/165), and hydrocodone-D6 (306/202) were monitored.

Results: The procedure was applied to 50 authentic urine specimens previously tested positive for two or more analytes using acid hydrolysis and GC/MS. Results showed that efficient hydrolysis is essential to the optimum recovery of all analytes. β-glucuronidase from both *H. Pomatia* and *E. Coli* were not able to cleave codeine glucuronides efficiently and recovered only 25% and 50% of the free drug after 3 hr and 16 hr hydrolysis time, respectively. On the contrary, 100% recovery was achieved for hydrocodone after 3 hr with both *H. Pomatia* and *E.Coli*. Average morphine recovery was 84% with *H. Pomatia* at 3 hr and 100% after 16 hr of incubation. *E. Coli* recovered 77% and 89% morphine at 3 hr and 16 hr, respectively. Average hydromorphone recovery with *H. Pomatia* was 80% after 3 hr and 95% after 16 hr. *E. Coli* recovered only 41% hydromorphone at the end of 3 hr and 58% after 16 hr of incubation. Doubling the amount of enzyme did not improve the recovery for any of the opiates.

Conclusion: Acid hydrolysis for opiates has been commonly used with GC/MS analysis in the past. With the advancement of instrumentation, LC/MS/MS is gaining popularity in the clinical and forensic labs and enzymatic hydrolysis is the preferred method for releasing the free drugs. Post-enzymatic hydrolysis specimens can simply be diluted and injected on the column, eliminating the need for time-consuming extractions. It is essential, however, to optimize the hydrolysis conditions for the opiate glucuronides specific to each source of β-glucuronidase. Codeine glucuronide is the most difficult to cleave and only 50% of the drug was recovered in free form after 16 hr of hydrolysis with β-glucuronidase from *H. Pomatia.* In general, the enzyme from *H. Pomatia* performed better than the one obtained from *E. Coli*, under the conditions tested. Although *H. Pomatia* was able to release 100% of the free drug form morphine, hydrocodone, and hydromorphone conjugates in the urine samples at the end of 16 hr (3 hr in case of hydrocodone), it was found to be ineffective in cleaving codeine glucuronide. Further investigation is necessary to find the optimal conditions for enzymatic hydrolysis of codeine. The labs must carefully evaluate the hydrolysis efficiency of various enzymes for opiates and specifically for codeine.

Opiates, Hydrolysis, LC/MS/MS

 ** Presenting Author*

K58 Sweat as Alternative Matrix to Monitor Buprenorphine Compliance, Opioids, Cocaine, and Tobacco Use in Opioid- Dependent Pregnant Women

Marta Concheiro-Guisan, PhD, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A729, Baltimore, MD 21224; Hendree E. Jones, PhD, Johns Hopkins Bayview Medical Center, 4940 Eastern Ave D 3 E, Baltimore, MD 21224; Rolley E. Johnson, PharmD, 10710 Midlothian Turnpike, Richmond, VA 23235; Robin Choo, PhD, National Inst on Drug Abuse, Chemistry and Drug Metabolism, 5500 Nathan Shock Dr, Baltimore, MD 21224; and Marilyn A. Huestis, PhD*, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Blvd, Rm 05A721, Baltimore, MD 21224*

The goal of this presentation is to describe Buprenorphine (BUP), opioids, cocaine, and tobacco prevalence and concentrations in sweat patches from opioid-dependent pregnant women, and to compare their detection in sweat patches, Oral Fluid (OF), and urine.

This presentation will impact the forensic science community by showing how sweat is a good alternative matrix for monitoring drug use in clinical settings.

Introduction: Sweat is an alternative matrix for detecting drug consumption over about seven days, depending upon the time the patch is worn. Sample collection is easy, gender-neutral, and less invasive than for urine collection. However, limited sweat disposition data are available, especially for BUP and for opioid-dependent women.

Objective: To describe BUP, opioids, cocaine, and tobacco prevalence and concentrations in sweat patches from opioid-dependent pregnant women, and to compare detection in sweat patches, OF, and urine specimens over the same period.

Methods: Sweat patches were collected once weekly (n = 121), and OF and urine twice or three times weekly (n = 283) from seven opioid-dependent pregnant women during the 2nd and, primarily, the 3rd trimester, and up to one month postpartum. Sweat was collected with PharmCheck™ sweat patches worn for 6 ± 2.3 days, and OF with the Salivette® collection device. Sweat and OF specimens were analyzed by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) for BUP, norbuprenorphine (NBUP), methadone, 2-ethylidene-1,5-dimethyl-3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), morphine, codeine, 6-acetylmorphine (6AM), heroin, 6-acetylcodeine (6AC), cotinine, and trans-3′-hydroxycotinine (OH-cotinine) (LOQ 1-5ng/patch, 0.5-1ng/mL, respectively). Urine specimens were assayed for cocaine and opiates by immunoassay (cutoff 300ng/mL). Women received 8 – 24mg BUP daily.

Results: BUP was detected in 88% of sweat patch specimens (median 2ng/patch; range 1 – 15.3ng/patch) and NBUP in 37.2% (range 1 – 24.7ng/patch). BUP alone was dectected in 51.2%, along with NBUP in 37.2%. Cotinine was detected in 89.3% (median 159ng/patch; range 8.9 – 1,390ng/patch) and OH-cotinine in 86% (median 52.5ng/patch; range 3.1 – 377ng/patch). Most OF specimens contained both analytes (86%). Methadone from non-prescribed sources was detected in 47.9% specimens (range 1 – 661ng/patch); and EDDP in 14.9% (range 1 – 18.4ng/patch). In 24% of specimens, 6AM was identified (range 1.2 – 180ng/patch), morphine in 23.1% (range 2.3 – 51.3ng/patch), heroin in 14% (range 1.1 – 526 ng/patch), codeine in 9.1% (range 4.5 – 25.1ng/patch), and 6AC in 8.3% (range 1.4 – 17.8ng/patch). Morphine and 6AM were detected alone (5% and 3.3%, respectively), together (5.8%), or in combination with the other analytes (6.6%). For identifying illicit cocaine exposure, cocaine was identified in 88.4% of specimens (median 14ng/patch; range 1 – 5,660ng/patch), BE in 47.9% (range 1.4 – 850ng/patch), and EME in 18.2% (range 8.7 – 567ng/patch). Cocaine was detected alone in 39.7% of cases, cocaine and BE in 30.6%, and the three analytes in 17.4%. Comparing sweat patches and urine, there was an 85.1% concordance for opioids, while for cocaine, only 35.9% agreement was achieved. Sweat patches and OF concordance was 93% for tobacco, 88.6% for BUP, 81.6% for opioids, 61.5% for methadone, and 56.1% for cocaine.

Conclusions: These results offer new information about drug and metabolite concentrations and prevalence in sweat from opioid-dependent pregnant women. Sweat is a good alternative matrix for monitoring drug use in clinical settings.

Sweat, Pregnant Women, Drug Testing

 ** Presenting Author*

K59 Discovery-Based Analyses of Wastewater Samples for Characterization of Drug Usage

Adrienne W. Brockman, BS, 107 Whitmore Labs, University Park, PA 16802; Jack Cochran, BS, 110 Benner Cir, Bellefonte, PA 16823; Michelle Misselwitz, BS, 110 Benner Cir, Bellefonte, PA 16823; and Frank Dorman, PhD, 107 Whitmore Labs, University Park, PA 16802*

After attending this presentation, attendees will understand the principles and design of Wastewater Treatment Facilities (WWTFs) and how these locations and wastewater systems in general may be used to determine temporal and spatial trends in the usage of a wide variety of compounds. Monitoring human usage of several categories of controlled substances can yield the information to allow for either targeted enforcement or targeted education. Additionally, the location of possible synthetic operations may be determined through the monitoring of wastewater. This may aid law enforcement in focusing their efforts in a certain region. Additionally, attendees will have a better understanding of appropriate extraction methods used to recover specific analytes from wastewater and also know what steps can be taken to identify and quantify the analytes of interest.

This presentation will impact the forensic science community by providing a mechanism to inform law enforcement and community leaders on community drug habits by the development of a method for monitoring wastewater. Questions such as the following can be answered using this approach: In what areas/neighborhoods are drugs being consumed? What drugs are being consumed? In what volumes are drugs being consumed and in what usage patterns? This could help pinpoint at-risk populations for drug abuse, which may help tailor the drug education curriculum in specified school districts. This could also help facilitate law enforcement in combating areas with identified drug usage.

The information obtained through the monitoring of WWTFs may be used for a variety of purposes. The objective of this research is to determine compounds present in wastewater samples specifically obtained from the Pennsylvania State University wastewater treatment plant, which is being used as a control facility to refine analytical methodology. Rather than beginning with a target compound approach, a discovery analysis approach was chosen to attempt to determine as many compounds as possible prior to any compound list restriction. The difficulty in this approach can be the resulting complexity of the analysis. For this reason, utilization of both Comprehensive Gas Chromatography coupled with Time-Of-Flight/Mass Spectrometry (GC x GC/TOF/MS) analysis and also High Performance Liquid Chromatography coupled with Time-Of-Flight/Mass Spectrometry (HPLC/TOF/MS) analysis were chosen for their inherent ability to characterize these potentially complex samples more successfully compared to other possible techniques. Several categories of compounds were found using this approach in the initial discovery experiments. Specifically, a number of antidepressants (SSRI's and MAOI's), synthetic opioids, and steroids, were found in addition to a number of endocrine disrupting compounds. Additionally, a series of chlorophenyl cyanates were found, which may indicate a chemical synthesis operation, though likely not an illicit drug facility. The determination, through spatially-resolved sampling, of the source will serve as a model for how other synthesis operations could be uncovered and located through the use of this procedure. An example of the complexity of the analysis is revealed through inspection.

Once the discovered compounds are identified and quantified, the ultimate goal is to determine when and where these compounds were introduced into the wastewater system. Employing time-resolved sampling at locations upstream from the WWTF, both the location and the usage patterns were narrowed down. Although a large array of compounds could be identified in the wastewater, this research project will focus particularly on drugs and drug metabolites.

For the discovery phase, samples of wastewater were gathered by "grab" sampling from the Penn State WWTF. Multiple four-liter samples were gathered from each of the following: influent flow, effluent flow, three intermediate stages, and final spray effluent. Following USEPA method 3510, a liquid-liquid extraction process was performed to demonstrate a "baseline" to compare with other extraction methods. A separatory funnel was used for extraction purposes, with methylene chloride as the solvent. Immediately following, the Kuderna-Danish technique was used to concentrate the samples to 1mL. Once the samples had undergone the clean-up process, they were introduced to the analytical systems to identify and quantify the compounds.

The presentation will discuss the methods used to extract, identify, and quantify the analytes of interest. Also, in the discussion, Pharmaceuticals And Personal Care Products (PPCPs) will be brought to the forefront for conversation, as this research project's focus also covers PPCPs as well as emphasizing drugs and drug metabolites.

Analysis, Wastewater, Drugs

 ** Presenting Author*

K60 Analysis of the Cocaine Metabolite Benzoylecgonine in Wastewater

Juliet Kinyua, MSc, and Todd Anderson, PhD, The Institute of Environmental and Human Health, Texas Tech University, 1207 Gilbert Dr, Lubbock, TX 79416*

After attending this presentation, attendees will gain insight on the utility of sewage epidemiology as a tool in forensic toxicology and the multiple applications of this methodology beyond the forensic science discipline, particularly in regard to toxicological investigations within law enforcement. The presentation will delineate the long-term benefits of this approach to society and law enforcement.

This presentation will impact the forensic science community by contributing an optimized method to forensic toxicology. Sewage epidemiology will reveal comprehensive information on the concentrations of illicit drugs in raw sewage that enables a more precise estimation of their illegal usage, and a relatively quick alternative to gain insight into the toxicological map of a given area. The presentation is geared toward generating interest in developing robust techniques for continuous data generation that can be used to make correlations with crime statistics and create sturdy monitoring tools.

Abuse of illicit drugs is a major problem in society and leads to high morbidity, mortality, and is responsible for many socio-economic problems. Estimates of cocaine consumption are currently obtained from crime statistics, population surveys, and consumer interviews; these estimation methods may not reflect the real extent of cocaine abuse. Another approach that has been used successfully in Europe is sewage epidemiology—a technique based on analysis of urinary biomarkers in sewage. This approach is based on analysis of a stable cocaine metabolite, Benzoylecgonine (BE) in waste water. In humans, cocaine is extensively metabolized to BE by chemical hydrolysis and Ecgonine Methyl Ester (EME) by enzymatic hydrolysis. BE is the major metabolite of cocaine; its presence in urine confirms cocaine abuse. In urine, cocaine can be detected up to 8 hr after use, while BE and EME can be identified for more than 96 hr after cocaine use.

Sewage epidemiology offers an adaptable, alternative method to consistently measure and monitor community drug use. Furthermore, the results from the study can be used to establish a framework for drug use monitoring. The goal of this study was to test the utility of sewage epidemiology in monitoring cocaine metabolite BE in waste water. Influent to the Lubbock (TX) Water Reclamation Plant (LWRP) was tested twice a week to assess weekly variations in cocaine consumption over a five-month period (February 2010 – June 2010). BE was extracted from influent wastewater samples using solid phase extraction and analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). Filtered 500ml influent waste water samples were spiked with deuterated internal standard (BE-d_3) and extracted using Oasis® MCX 60mg SPE cartridges. To determine BE and BE-d_3 in sample extracts using GC/MS, the extracts were first derivitized using N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) to enhance volatility forming the Trimethylsilyl (TMS) derivative of BE or BE-d3. GC/MS analyses was performed using a DB-5MS column for separation and the Mass Selective Detector (MSD) was set to operate under selected ion monitoring mode targeting ion masses of 82, 240, and 361 for BE and 243 and 364 for BE-d_3.

The concentrations of BE derived from the analysis were used to calculate cocaine equivalents deposited in the sewer system through excretion by users. The cocaine equivalents and wastewater daily volumes and flow rates were used to estimate cocaine use by the population. The average daily consumption of cocaine during the study period was estimated at 1,152 ± 147g. Higher cocaine consumption was observed on weekends compared to weekdays. The present study showed that sewage epidemiology is a useful tool to detect BE and subsequently estimate cocaine consumption. The method described is an efficient tool for investigating temporal variations (daily, weekly, and seasonal) at a local level. In addition, this method, along with the ability to sample wastewater at the neighborhood level, could provide a valuable forensic tool for law enforcement.

Cocaine, Sewage-Epidemiology, Toxicological Map

 ** Presenting Author*

K61 Weeding Analytes Out of Marijuana: The Identification and Quantification of Pesticides in Cannabis Utilizing Comprehensive Gas Chromatography

Emily Ly, and Frank Dorman, PhD, 107 Whitmore Labs, University Park, PA 16802; and Jack Cochran, BS, and Julie Kowalski, BS, 110 Benner Cir, Bellefonte, PA*

After attending this presentation, attendees will understand the principles of analyzing and quantifying cannabis for specific cannabinoids such as Cannabinol (CBN), Cannabidiol (CBD), Cannabichromene (CBC), cannbigerol (CBG), and Delta-9-Tetrahydrocannabinol (THC) using Gas Chromatography with Flame Ionization Detection (GC/FID). They will also understand the concepts and reasons for testing for pesticides contained on cannabis utilizing Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Electron-Capture Detectors (GC/ECD) as well as the practical applications for such analyses. Finally, attendees will understand how Comprehensive Gas Chromatography (GCxGC) can be utilized to potentially determine both potency as well as trace organics characterization in a single analysis.

This presentation will impact the forensic science community in a variety of different ways, but loosely falls into two classes: characterization of cannabis as a potential pharmaceutical; and, potentially fingerprinting the trace compounds in cannabis to determine the point of origin. Testing for potency can help determine the identity and abundance of target cannabinoids that have therapeutic qualities. These qualities have been confirmed to relieve pain, control nausea, stimulate appetite, and decrease ocular pressure.[1] With this knowledge, medical marijuana can be grown more effectively by lessening the main psychoactive component, THC, which may cause discomfort in patients, and increasing the target therapeutic cannabinoids.[2] The abundance of THC and other cannabinoids is affected by a variety of factors including environmental conditions, harvesting periods, and the sex of the plant.[3] Furthermore, many pesticides, fungicides, and insecticides are used to treat the cannabis plant. This is of concern for any person that consumes the material due to the residual toxins that are potentially harmful. Moreover, a study was conducted in 1992 for the United States Drug Enforcement Administration (DEA) which determined that chemical profiles of cannabis samples could be used to locate the geographical origin.[4] However, the system could only eliminate possible sources of origin and therefore had low specificity due to the fact that only cannabinoid constituents were analyzed.[4] By identifying and quantifying the pesticides on the cannabis plant, it may be possible to develop a “chemical fingerprint” relating to compounds used by growers to increase the crop yield. This information may allow law enforcement agencies determine and/or link the source location of the confiscated illicit drug.

One hundred and six different samples of illicit marijuana were analyzed. These were obtained directly from local law enforcement personnel. Samples were initially homogenized, and the finely ground marijuana, weighing approximately 0.2 to 2g, was mixed with 10mL of acetonitrile and 10mL of water in 50mL centrifuge tubes, similar to the QuEChERS extraction procedure developed at the USDA.[5] Water was added to increase the extraction efficiency of more polar pesticides.[6] This solution was spiked with internal standards and pesticides for recovery purposes before soaking for an hour. The solution was shaken for 30 min with a vortex mixer. QuEChERS EN salts were added and the solution was shaken for 1 min.[5] This was followed by a 5 min phase separation utilizing centrifugation.[5] The supernatant was removed and refrigerated.[6] QuEChERS extraction is an efficient method that minimizes organic solvent waste and increases laboratory throughput as compared to more conventional solvent extraction techniques. SPE clean-up followed the extraction step to remove high levels of chlorophyll and organic acids that may interfere with the resulting chromatographic analysis.[6] Sample extract clean-up was performed by cartridge SPE procedures utilizing a 500mg graphitized carbon black/500mg Primary Secondary Amine cartridge, to which MgSO4 was added to the top of the SPE cartridge at approximately half the height of the GCB/PSA bed. The cartridge was rinsed with 20mL of acetone. Then 0.5mL of the sample extract was added, 2.5uL of anthracene (recovery surrogate) was spiked onto the cartridge and carefully mixed by syringe. The solution was eluted with a 3:1 acetone:toluene mixture. The solution was then evaporated with nitrogen at 108°C until it was reduced to approximately 0.3mL. Toluene was added to adjust the final volume to 0.5mL. The samples were analyzed using various GC methods, which will be discussed in detail during the presentation, allowing for the potency analysis and the pesticide fingerprint to be determined in a single GCxGC separation.

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Cannabinoids, GC/MS, GC-FID

 ** Presenting Author*

K62 Forensic Toxicology Findings in 150 Alleged Cases of Drug-Facilitated Sexual Assault (DFSA) in San Francisco

Nikolas P. Lemos, PhD, Eric A. Ingle, BA, Cecilia O. Medina, BS, Glenda M. Easterling, BS, Pavlos Karamanidis, BS, Chinyere M. Williams, BS, Jonas E. Knight, BS, Jonas E. Knight, BS, and Justin A. Volk, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103*

After attending this presentation, attendees will understand the wide variety of drugs commonly encountered in DFSA cases and value the usefulness of toxicology testing in such cases.

This presentation will impact the forensic science community by providing valuable information on drug incidence in victims of DFSA and will offer blood reference concentrations of drugs commonly detected in such cases.

Between July 1, 2010, and June 30, 2011, the Laboratory Division of the San Francisco OCME performed toxicologic evaluations in 150 cases of suspected Drug Facilitated Sexual Assault (DFSA). The age of the subjects ranged from 12 to 57 years (mean: 30). The 150 cases were comprised of 129 females, 18 males, and three transgender females. Healthcare providers attending to DFSA victims are trained to collect urine specimens in these types of cases and only collect blood specimens if the alleged crime took place within hours of their examination. Specimens are typically evaluated for ethanol and related volatiles using Gas Chromatography equipped with Flame Ionization Detection (GC/FID), then screened by Enzyme-Linked Immunosorbent Assay (ELISA) for amphetamines, benzodiazepines, cannabinoids, cocaine, methadone, phencyclidine (PCP), ketamine, and opiates/opioids and by Gas Chromatography/Mass Spectrometry (GC/MS) for over 100 other drugs and metabolites including, but not limited to, diphenhydramine, carisoprodol, meprobamate, and γ-hydroxybutyrate (GHB).

Of the 150 cases, 101 had urine only, 17 had blood only, one had plasma only, and 31 had both blood and urine specimens. The 101 cases with only urine pertained to 93 females, five males and three transgender females. Of them, 33 cases had no drugs detected, but 28 were positive for ethanol with a mean ethanol concentration of 0.22% (w/v) and a range of 0.04 to 0.44 % (w/v). It is noteworthy that ethanol is only quantified in urine if the Division is provided with a forensic urine specimen (i.e., a urine specimen obtained about 20 min after the voiding of the urinary bladder; a collection protocol believed to produce urine that is a recent kidney filtrate and which better approximates blood ethanol concentrations). Besides ethanol, the most frequently reported substances in these urine specimens were cocaine/benzoylecgonine (15/18 cases), THC-COOH (14 cases), levamisole (11 cases), diphenhydramine (10 cases), and methamphetamine/amphetamine (8/8 cases). Many other psychoactive compounds were also detected as listed in Table 1.

The 18 cases which had blood products (17 whole blood specimens and one plasma specimen) pertained to 10 females and 8 males. These alleged victims' age averaged 27 years (range: 12 – 41 years). The plasma case was found to be negative. Ethanol was reported in five cases with a mean concentration of 0.11% (w/v) and a range from 0.01 – 0.15% (w/v). Methamphetamine was the most commonly encountered substance in these blood cases as it was found in four cases with a mean concentration of 0.35mg/L (range: 0.16 – 0.66mg/L). Amphetamine was detected in two blood specimens (0.01 and 0.04mg/L) as was THC-COOH (6 and 86ng/mL). Finally, THC was found in one of these blood specimens at a concentration of 8ng/mL.

The 31 cases that had both blood and urine specimens associated with them pertained to 26 females and five males whose average age was 29 years (range: 17 – 57 years). Of these 31 cases, seven had no drugs detected in either blood or urine. Ten blood specimens were positive for ethanol at a mean concentration of 0.11% (w/v) with a range of 0.02 – 0.37% (w/v). THC and THC-COOH were the most commonly encountered substances in these blood specimens found in six and four cases, respectively. THC mean concentration was 2ng/mL with a range of 1 – 4ng/mL while THC-COOH mean concentration was 20ng/mL with a range of 6 – 59 ng/mL. Other psychoactive compounds were also detected in these blood specimens as presented in Table 2. Of the corresponding 31 urine specimens, nine were positive for ethanol at a mean concentration of 0.17% (w/v) with a range of 0.02 – 0.38% (w/v). Methamphetamine, amphetamine, diphenhydramine, THC-COOH, and cocaine were among the most frequently detected drugs in this set of urine specimens (Table 2).

This study demonstrates the variety of substances that are commonly encountered in alleged DFSA victims' toxicology specimens. Ethanol, cocaine, methamphetamine, cannabis, and diphenhydramine are among the most frequently encountered drugs in DFSA case investigations. Interestingly, 44% of the cases reported early enough for a blood collection to take place pertained to male victims, suggesting males are more likely to report early on to the authorities that they may be victims of DFSA but females often delay the reporting, thus rendering blood collection useless. This study will improve the ability of forensic toxicologists and law enforcement personnel to better participate in the investigation of such crimes in their own jurisdictions.

DFSA, Toxicology, San Francisco

 ** Presenting Author*

K63 Epidemiology of Rodenticide Poisoning in Manipal, South India

Shankar M. Bakkannavar, MD, Dept of Forensic Medicine, Kasturba Medical Coll, Manipal, 576104, INDIA; Pradeep K. Gouda, MD, Kasturba Medical Coll, Manipal Univ, Manipal, 576104, INDIA; and Vinod C. Nayak, MD, Dept of Forensic Medicine, Kasturba Medical Coll, Manipal, 576104, INDIA*

After attending this presentation, attendees will gain knowledge about the incidence and prevalence of rodenticide poisoning cases in South India. The toxicoepidemiology of the rodenticide poisoning, will be presented. Attendees will better understand how the hemorrhagic manifestations appear on the body, and the proper diagnosis can be better understood.

This presentation will impact the forensic science community by providing information about hemorrhagic manifestations occurring in rodenticide poisoning and the duration of their occurrence. This research provides more information in an area with little previous research. This presentation will add to research being carried out in forensic medicine by broadening the understanding of how hemorrhagic manifestations occur in rodenticide poisoning cases, enabling a better appreciation of these manifestations in humans.

Human Poisonings due to chemicals like insecticides, rodenticides, etc. commonly occur because of easy accessibility. Poisoning due to rodenticides, even though rare, are not uncommon. The mortality and morbidity due to rodenticides is increasing worldwide. Hence, knowledge about the epidemiology and clinical manifestations of rodenticide poisoning is not only essential for the treating doctor, but also to the forensic pathologist. The hemorrhagic manifestations of rodenticide poisoning can sometimes mimic contusions, posing problems while interpreting the injuries.

In this retrospective research, the toxic epidemiology of fatal poisoning due to rodenticides in this part of the world is described. The hemorrhagic manifestations and altered laboratory findings in the victims will also be discussed.

In the present study, fatal rodenticide cases constituted 13.89% of the total poisoning cases, with the majority of the victims being male. The age of the victims ranged from 2 to 82 years. External hemorrhages were present in only five cases, although hemorrhage in Gastrointestinal Tract (GIT) was seen in a maximum number of victims. The prothrombin time (PT) was increased in 21 cases. The enzymes such as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were raised in 20 cases and total bilirubin was raised in 13 cases.

Rodenticides belong to a category of pest control chemicals intended to kill rodents. The chemicals which act as rodenticides are anticoagulants like warfarins, superwarfarines, thallium, phosphorous, metal phosphides, barium carbonate, red squill, strychnine, etc. Though the mechanism of action of various rodenticides is different, all lead to coagulopathy. Various studies done in the past have suggested the hemorrhagic manifestations due to altered coagulation profiles were induced by these rodenticides.[1-3] Substantial ingestion produces epistaxis, gingival bleeding, widespread bruising, hematomas, aematuria with flank pain, menorrhagia, gastrointestinal bleeding, rectal bleeding, and hemorrhage into any internal organ. Spontaneous hemoperitoneum has been described. Severe blood loss may result in hypovolaemic shock, coma, and death.[1] Hematoma and hemarthrosis as reported by Greeff, M.C., *et al*. were observed in children who accidentally consumed redenticide.[4] Cutaneous hemorrhage and hematemesis were also observed by Dolin E *et al*. in their study.[5] Similar observations were made in this study also.

In conclusion, hemorrhagic manifestations as a result of rodenticide poisoning can be misinterpreted as being due to assault. The differential diagnosis is quite broad and includes all causes of vitamin K deficiency, Disseminated Intravascular Coagulation (DIC), and liver disease. Perusal of hospital records is highly recommended for coagulation profile and is of paramount importance while concluding mode, manner, and cause of death. Rodenticide poisoning as a probable cause should be considered in nontraumatic bruises associated with suspected poisoning cases encountered at autopsy.

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Epidemiology, Rodenticide, Hemorrhages

 ** Presenting Author*

K64 Stability of Seven Benzodiazepines Together With Zolpidem, Methodone, and Propoxyphene in Bloodstains

Carole Jamey, Antoine Tracqui, MD, PhD, Bertrand Ludes, MD, PhD, and Jean-Sébastien Raul, MD, PhD, Institut de Medecine Legale, 11 Rue Humann, Strasbourg, 67085, FRANCE*

After attending this presentation, attendees will learn about another aspect of bloodstain analysis. The goal of this presentation is to emphasize the potential interest in toxicological analysis of bloodstains.

This presentation will impact the forensic science community by highlighting another aspect of bloodstain analysis that should be valued in forensic practice.

Blood ranks among the most usual kind of physical evidence encountered on a crime scene. Individualization of human blood has been performed for decades by using the ABO system and, more recently, DNA typing granted the forensic scientist a high-performance tool for this purpose. Forensic toxicology, however, also followed a continuous progress, currently providing the possibility to detect various drugs in very small blood samples. This purportedly offers the opportunity to assay bloodstains for toxicological analysis, which could be of interest in some situations, e.g., determination of the victim's toxicological status even if no corpse is found at the crime scene, or of the perpetrator's status if he/she bled in the surroundings of the crime scene. Moreover, when DNA typing cannot be compared to a reference (the victim and/or any biological element for DNA comparison was not found), detecting drugs in bloodstains could contribute to the victim's identification. Until now, only a few works have dealt with the detection of drugs in such samples, and the stability of drugs in bloodstains under different storage conditions have never been studied before. The stability of seven benzodiazepines (diazepam, bromazepam, clonazepam, alprazolam, clobazam, tetrazepam, and triazolam) were investigated, together with zolpidem, methadone, and propoxyphene over periods ranging from 24 hr to one month under various environmental conditions (drugs were chosen because of their frequent prescription in France).

Drug-free 50µL blood samples were spiked with an amount of 10ng/ml of each analyte (500pg per blood sample) and deposited on a glass slide. After storage at -20°C, +4°C, and +35°C away from light, at +20°C in daylight, away from light, and in "extreme" conditions (outside the laboratory exposed to daylight, wind, and variable temperature), bloodstains were collected after 24hr, 48hr, 72hr, one week, and one month by scratching and by swabbing. When scratched, the bloodstain was weighed and rehydrated for 45 min in 0.5mL ammonium buffer (pH 9.5). Swabbing was performed with swabs previously moistened with saline. Swabs were placed in 0.5mL ammonium buffer, sonicated 15 min, and stored away from light for 45 min before swabs were removed. Liquid/liquid extraction was performed using methylenechloride, N-Heptane, isopropanol (65:25:10, v/v) with Prazepam as an internal standard. Then toxicological analyses were carried out by Ultra Performance Liquid Chromatography/Liquid Chromatography with Tandem Mass Spectrometry (UPLC/MS/MS) under previously reported conditions.

The validation of the method was performed on dried bloodstains stored away from light at room temperature for six hours spiked with the different analytes. Under these analytical conditions, the method appeared sensitive (0.0005<LOQ<0.005ng/mg), linear (LOQ-10ng/mg), and accurate (CV<20%).

Results showed a good stability of all drugs tested even after one month of storage in each condition, except for clonazepam at -20°C (sometimes undetected), and for all drugs tested at +35°C and in "extreme" condition with sometimes up to 50% loss after one month. By scratching or swabbing, each analyte could be detected, except for clonazepam at -20°C. This study shows an acceptable stability of most benzodiazepines, zolpidem, methadone, and dextropropoxyphene in dried bloodstains. It opens the way to a new analytical approach which may enhance the bloodstain pattern analysis of a crime scene.

Bloodstain, Drugs, Stability

 ** Presenting Author*

K65 Determining Zolpidem Compliance: Urinary Metabolite Detection and Prevalence in Chronic Pain Patients

David M. Schwope, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228; Anne DePriest, PharmD, 515 Great Circle Rd, Nashville, TN 37228; David L. Black, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228; Edward J. Cone, PhD, Cone Chem Research, LLC, 441 Fairtree Dr, Severna Park, MD 21146; Yale H. Caplan, PhD, National Scientific Services, 3411 Philips Dr, Baltimore, MD 21208-1827; and Rebecca Heltsley, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228*

After attending this presentation, attendees will be able to describe a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for the simultaneous identification and quantification of zolpidem (Ambien®) and its primary urinary metabolite, zolpidem 4-phenyl carboxylic acid (ZCA), in human urine.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific simultaneous quantification of zolpidem parent and metabolite in a single urine extract, as well as providing useful data detailing zolpidem metabolite prevalence in a chronic pain patient population.

Introduction: Zolpidem is the most prescribed insomnia treatment in the United States; however, little is known about zolpidem metabolite excretion in chronic pain patients. As zolpidem is extensively metabolized *in vivo*, metabolite detection may provide improved accuracy for compliance determinations, thereby improving clinical decisions and treatment courses. It is believed that no reported method simultaneously quantifies both parent zolpidem and ZCA in urine.

Method: This study was IRB-approved. Zolpidem and ZCA were extracted from 1mL human urine by mixed-mode solid-phase extraction following buffering with 0.1M acetic acid. Samples were eluted, evaporated to dryness, and reconstituted in 200µL aqueous mobile phase. Samples were injected onto an LC/MS/MS instrument comprised of a Shimadzu Prominence HPLC and ABSciex™ API 3200 tandem mass spectrometer. Ionization was by electrospray (positive mode) with Multiple Reaction Monitoring (MRM) mode employed for detection and quantification. Gradient chromatographic separation starting at 20% B (0.1% formic acid in acetonitrile) was achieved using a C_{18} column (100 x 2.1mm, 3µm particle). Flow rate was 0.7mL/min with an overall run time of 1.8 min.

Results: Conservative Limits Of Quantification (LOQ) were 4ng/mL for both analytes. The assay was validated for linearity from 4 – 1,000ng/mL for zolpidem and 4 – 5,000ng/mL for ZCA (r^2>0.990 and concentrations within ±15% of target). Inter-day recovery (bias) and imprecision (n=20) were 100% – 107% of target and 2.4% – 3.7% relative standard deviation, respectively. Extraction efficiencies were 78% – 90%. Freeze-thaw, processed sample, and autosampler stability were examined (n=6 each), with concentration changes <6.0% observed in all cases. No quantifiable carryover was observed at the method Upper Limit Of Quantification (ULOQ).

A total of 3,264 urine samples were obtained from chronic pain patients over five months and analyzed, with 3,142 (96.3%) meeting qualitative acceptance criteria. Results were de-identified and examined for zolpidem and ZCA prevalence, with concentrations normalized to urine-specific gravity. Zolpidem was detected > LOQ in 720 specimens (22.9%) while ZCA was detected in 1,579 specimens (50.3%). Two specimens (0.06%) contained zolpidem > ULOQ and 45 specimens (1.43%) contained ZCA > ULOQ. Of specimens within the dynamic linear range, median (range) zolpidem and ZCA concentrations were 28.3 (4.08 – 805) ng/mL and 2,038 (4.53 – 23,000) ng/mL, respectively. Only five specimens (0.16%) contained zolpidem alone (median concentration 488ng/mL). As ZCA was observed without parent zolpidem in 864 samples, addition of this metabolite to the assay increased detection rates by 27.5% in this cohort.

Conclusions: An LC/MS/MS method for simultaneous detection and quantification of zolpidem and ZCA in human urine is presented. Addition of zolpidem metabolite to compliance determinations resulted in substantially more positive samples compared to zolpidem alone at the same LOQ. This method is rapid and conducive to a high-throughput environment. Improved detection windows for zolpidem intake should prove useful in both clinical and forensic settings.

Zolpidem Metabolite, Compliance, LC/MS/MS

 ** Presenting Author*

K66 Detection of Volatiles in Postmortem Samples by Headspace Gas Chromatography With Mass Spectrometry

Charlotte A. Baker, PhD, 2212 Pearl Bay Ct, Pearland, TX 77584; Fessessework Guale, DVM, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054; Ashraf Mozayani, PhD, PharmD, Texas Southern Univ, 3100 Cleburne Ave, Houston, TX 77004; and Jeffrey P. Walterscheid, PhD, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054*

After attending this presentation, attendees will become knowledgeable about the detection of toxic volatiles in postmortem specimens such as blood, lung, and brain using Headspace/Gas Chromatography with Mass Spectrometry (HS/GC/MS). Four cases of death caused by toluene, difluoroethane, difluorochloromethane, and nitromethane abuse will be discussed.

This presentation will impact the forensic science community by providing a more reliable method for the detection of volatiles in postmortem specimens.

Volatile inhalants have become a common and dangerous substance of abuse. These inhalants include a broad range of chemicals such as volatile organic solvents, aerosol propellants, and other gases which are readily available, easily purchased, and can be used without supervision or accessories. The mechanism of acute intoxication and death due to inhalant abuse is through fatal ventricular arrhythmia, asphyxiation, and pulmonary edema.

Undoubtedly, the ability to confirm these gases in autopsy specimens presents unique challenges to the forensic toxicologist. Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) is currently the most widely applied technique in determining the presence of volatile compounds. However, this method does not provide sufficient data for identifying poorly combustible gases. In these cases, HS/GC/MS is more advantageous because it provides spectral data specific to the volatile compound that can be matched with data stored in the NIST library for identification and confirmation. Postmortem samples such as blood, brain, and lung are collected by the medical examiners in a headspace vial and analyzed by HS/GC/MS. The analysis of these cases was performed using an Agilent 7890 GC equipped with Agilent 5975 inert, triple axis Mass Selective Detector (MSD) utilizing a split injection of 100:1. The column was DB-VRX (40m x 0.180mm x 0.100µm) and helium was used as the carrier gas. The oven was programmed for an initial temperature of 55°C that ramped to a final 80°C at a rate of 20°C/min and the entire run time was 11.25 min.

Case 1: Presents a 58-year-old White male found dead with evidence of spray paint on the fingers and face; spray paint cans were located at scene. Upon analysis, toluene was found in his blood sample. Toluene, or methylbenzene, is used in the production of benzene, solvent-based cleaning agents, household aerosols, nail polish, paints and paint thinners, lacquers, adhesives, and as a gasoline additive.

Case 2: Presents a 50-year-old female found with a spray can of Dust Off™ in her hand. Dust Off™, the main ingredient being difluoroethane, is becoming increasing favorable to inhale.

Case 3: Presents a 39-year-old White male found dead, lying prone beside an air conditioning unit, with his mouth against a pipe connected to the unit. Diflurochloromethane, also known as Freon, is growing in popularity because of its ease in being inhaled from outdoor units.

Case 4: Presents a 56-year-old White male that involves nitromethane, which is a component of airplane fluid. In this unique case, the decedent died as a result of consuming airplane fluid in an attempt to get drunk.

Volatiles, Headspace Gas Chromatography, Postmortem

 ** Presenting Author*

K67 Fatal Intravenous Injection of Oral Therapeutic Drugs in an Elderly Patient

Silvia Tambuscio, MD, Piazza De Gasperi 12 35131, Via Ospedale Civile 6 35121, Padova, ITALY; Luca Massaro, MD, Via degli Artigiani n 4, Este (PD), 35042, ITALY; and Giampietro Frison, PhD, Piazzale Giustiniani 11e/2, Mestre Venezia, ITALY*

After attending this presentation, attendees will be more conscious of the potential lethal effects following adverse reactions due to erroneous intravenous administration of oral therapeutic drugs.

This presentation will impact the forensic science community by promoting awareness of rare lethal therapeutic errors.

A 77-year-old male resident in a retirement home, suffering cardiac failure, severe neurological diseases, and dysphagia, died immediately after intravenous administration of a mixed compound, obtained by manual fragmentation of one tablet Respicur® 200mg (theophylline), one tablet of Dintoina® 100mg (phenytoin sodium salt), one tablet Luminale® 100mg (phenobarbital sodium), and a few milliliters of water. The nurse, educated in an Eastern European country and recently employed in Italy, intentionally injected the compound into a jugular catheter to bypass daily difficulties in oral administration, since the patient's parents had refused gastrostomy. A few minutes after the drug-blend injection, the patient showed convulsions, loss of consciousness with cardiac arrest.

Autopsy showed myocardiosclerosis and previous myocardial infarction, pulmonary emphysema, exogenous lipid pneumonia, interstitial fibrosis due to previous repeated gastric aspirations, remarkable congestion in the residual alveolar septa, and no emboli in the pulmonary vessels.

Toxicological analyses on the jugular catheter and syringe revealed extremely high concentrations of all three drugs, as expected from the unusual administration procedure. Toxicological analyses on biological specimens showed drug levels below the maximum therapeutic concentration (left ventricular blood: phenobarbital 15.33mcg/mL; theophylline 1.97mcg/mL; phenytoin 3.70mcg/mL; right succlavian artery blood: phenobarbital, 18.60mcg/mL; theophylline, 2.92mcg/mL; phenytoin, 5.26mcg/mL; left jugular cath residual blood: phenobarbital, 492.3mcg/mL; theophylline, 1395mcg/mL; phenytoin, 463.3mcg/mL). In fact, some degree of postmortem redistribution is expected to have occurred, considering the autopsy was performed three days after death.

Death was caused in this elderly patient by acute phenobarbital, phenytoin, and theophylline toxicity, following erroneous intravenous administration of oral therapeutic doses.

Severe theophylline-related arrhythmias happened very quickly after inoculation of the drug-blend, since the immediate and complete bioavailability produced extremely high concentrations, incomparable to any model of toxic overdose. The total lack of drug metabolism due to first hepatic passage was also responsible for this huge concentration. Moreover, intravenous injection at the left jugular site realized an exceptional condition inoculating theophylline very close to the heart, target of toxicity, and obtaining a sort of topic toxic effect at dramatically high concentrations.

The unusual way of administration also suggests phenytoin-related arrhythmias, as discussed for theophilline. Phenytoin is an anticonvulsant drug usually administered in tablets for chronic therapy, and intravenously at higher dosages for the treatment of acute epileptic seizures. In these cases, rapid parenteral injection can induce cardiac arrhythmias, while oral overdoses usually produce only neurological toxic effects.

Theophylline dose-related toxicity on the central nervous system should also be considered in the mechanism of lethality. On the contrary, the role of phenobarbital neurotoxicity is ruled out, since death rapidly took place and this barbiturate needs a longer time span to cross the hematoencephalic barrier.

Other possible lethal effects related to chemico-physical properties of excipients contained in the micro-fragments of injected tablet-mixture have been considered, but no relevant toxicological or hystopathological findings were noted.

In conclusion, this is the first case in the forensic literature reporting fatality by erroneous parenteral administration of oral therapeutic drugs. This study also points out that in similar occurrences postmortem analytical data have limited value. For this reason, the forensic scientist takes advantage in the diagnosis by the application of uses an appropriate methodology evaluating a complex of elements (circumstantial data; pathology; analytical data), but mainly inquiring/discussing evaluating the pharmacodynamic of each drug in relation to this bizarre irregular method of administration.

Teophylline Toxicity, Phenytoin Toxicity, Therapeutic Error

 ** Presenting Author*

K68 Postmortem Redistribution and Necrokinetics of Amphetamine, Cocaine, Morphine, and Oxycodone During Post- Embalming Decomposition

Murray K. Marks, PhD, Univ of Tennessee, Dept of Pathology, 1924 Alcoa Highway, Box 108, Knoxville, TN 37920*

WITHDRAWN

K69 Cannabinoids in 105 Postmortem Forensic Toxicology Cases

Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103*

After attending this presentation, attendees will understand the usefulness and value of postmortem cannabinoid analysis and will have a better understanding of the potential cardiotoxic effects of these compounds in humans.

This presentation will impact the forensic science community by providing postmortem cannabinoid incidence data among different types of deaths and by offering postmortem cannabinoid blood reference concentrations.

Between July 1, 2010, and June 30, 2011, the laboratory performed 1,338 postmortem toxicologic evaluations. Cannabinoids were confirmed/quantified in 105 cases (7.8%) comprising of 31 naturals, 31 homicides, 30 accidents, 12 suicides, and one undetermined. Decedents averaged 40 years (range: 16 – 71) and were predominantly male (72%).

Of the 31 naturals, one had antemortem whole blood (AMB) where only THC-COOH was found (11ng/mL). Twenty-eight had peripheral blood (BLP) where THC (n=25; mean: 8; range 1 – 48ng/mL), THC-COOH (n=22; mean: 75; range 5 – 640ng/mL), and THC-OH (n-=4; mean: 77; range: 17 – 210ng/mL) were found. One natural had central/cardiac blood (BLC) and only THC-COOH was detected (69ng/nL). The final natural case involved a decomposed decedent with no blood but THC-COOH was measured in her liver (2.6mg/kg).

In the 31 homicides, four cases had AMB and THC (n=3; mean: 2; range 1 – 5ng/mL), THC-COOH (n=4; mean: 31; range 10 – 78ng/mL), and THC-OH (n=1; 6ng/mL) were detected. Seventeen had BLP and THC (n=15; mean: 21; range 1 – 120ng/mL), THC-COOH (n=15; mean: 39; range: 1 – 170ng/mL), and THC-OH (n=2; 6 and 14ng/mL) were detected. Nine of the homicides had BLC and THC (n=5; mean: 7; range: 1 – 21ng/nL), THC-COOH (n=9; mean: 24; range: 7 – 96ng/mL), and THC-OH (n=1; 6ng/mL) were detected. The final homicide involved a decomposed decedent with no blood but THC-COOH was measured in her liver at a concentration of 1.4mg/kg.

In the 30 accidents, BLP and BLC existed in 27 and 3 cases, respectively. THC (n=20; mean: 6; range: 1 – 27ng/mL), THC-COOH (n=25; mean: 35; range: 5 – 330ng/mL), and THC-OH (n=1; 5ng/mL) were detected. In cases with BLC, THC (n=1; 3ng/mL) and THC-COOH (n=3; mean: 39; range: 5 – 89ng/mL) were found.

In the 12 suicides, only THC (n=8; mean: 7; range 2 – 24ng/mL) and THC-COOH (n=9; mean: 23; range 6 – 51ng/mL) were detected. Finally, in the one undetermined case, THC and THC-COOH were measured in BLP at 6 and 13ng/mL, respectively.

Comparison of postmortem BLP cannabinoid concentrations among types of cases suggest that THC-COOH averages the highest in natural deaths (75ng/mL, which is more than double its concentration in any other manner of death) whereas THC concentrations run on average three times higher in homicides (21ng/mL) than in any other manner of death. THC-OH, most often found when cannabis-containing products are eaten, averaged 77ng/mL in natural deaths as compared to only 10 and 5ng/mL in homicides and accidents, respectively.

Cannabinoids were not listed in the Cause of Death (COD) in any of the 31 homicides or in any of the 31 natural deaths, but featured as a Significant Other Condition (SOC) in 12 of the homicides (39%) and in 23 of the naturals (74%). Cannabinoids were listed in the COD in 8 of the 30 accidents (27%) and listed as an SOC in an additional 12 of these cases (40%). Cannabinoids were listed in the COD in one of the 12 suicides (8%) and were listed as an SOC in five more suicides (42%). Finally, the one undetermined death listed cannabinoids in the COD together with morphine.

Closer examination of the 31 natural deaths suggests that 84% of these (n=26) showed significant cardiac pathology such as hypertensive heart disease, atherosclerotic cardiovascular disease, and cardiomegaly. In three of these cases, the medical examiner was of the opinion that the decedent had suffered probable lethal cardiac arrhythmia which has previously been reported in the clinical literature as a possible toxic manifestation of cannabis overdose.

This study is the first of its kind and demonstrates the usefulness of cannabinoid analyses as part of every death investigation and provides postmortem concentration reference data that will improve the ability of toxicologists, medical examiners, coroners, and others to evaluate cannabinoid concentrations in human postmortem specimens as well as their possible contribution to death.

Cannabinoids, Forensic Toxicology, Cardiotoxicity

 ** Presenting Author*

K70 Tapentadol in Postmortem Casework

Ruth E. Winecker, PhD, and Justin O. Brower, PhD, OCME, Box 7580, Chapel Hill, NC 27312; and Laura W. Friederich, BS, 613A Pleasant Dr, Durham, NC 27703*

After attending this presentation, attendees will be able to detail the types of postmortem casework associated with tapentadol at various concentrations.

This presentation will impact the forensic science community by providing information regarding tapentadol as it relates to cause and manner of death determinations.

Tapentadol (Nucynta®, Palexia®, Zyntap®) is a mu-opioid receptor agonist and norepinephrine reuptake inhibitor. Approved by the Food and Drug Administration in 2009, tapentadol is available as immediate-release tablets of 50, 75, and 100mg and is indicated for acute moderate to severe pain. Adverse reactions associated with tapentadol are generally related to CNS depression including drowsiness, dizziness, headaches, as well as nausea and vomiting. Limited information has been published on tapentadol toxicity. A literature review indicates there are presently only four reported deaths in which the causative agent(s) included tapentadol.

At the North Carolina Office of the Chief Medical Examiner, cases suspicious for toxicological cause or with essentially negative autopsy findings are routinely screened for common over-the-counter, prescription, and illegal drugs via various laboratory techniques. This presentation will detail a group of 12 cases were tapentadol was detected during routine postmortem drug screening in support of cause and manner of death determination. Tapentadol is easily detected by the laboratory's basic organics screen which utilizes both Gas Chromatography with Nitrogen Phosphorus Detection (GC/NPD) and Gas Chromatography Mass Spectrometry (GC/MS). The extraction procedure has been previously described.[1]

Quantification of tapentadol is accomplished using GC-NPD with a calibration curve (blood matrix) and matrix matched positive/negative controls using the extraction procedure referenced above. Linearity, LOD, and LOQ are 0.2-2, 0.025 and 0.2mg/L, respectively. Accuracy and precision in blood (liver) are 99.4 (106) and 4.5 (12)%, respectively. Decedents were divided into groups according to manner of death for the purposes of studying tapentadol concentrations in overdose and non-overdose situations. The accidental and suicidal overdoses were subsequently divided into subgroups for further study: those where tapentadol was determined to contribute to the cause of death (attributed) and those where it was not (unattributed). The deaths in which tapentadol was determined to contribute to the cause of death were further divided into those where tapentadol additively combined with other drugs to cause the death and those where the drug was present in sufficient amounts to have caused the death regardless of other drugs and their concentrations.

Discussion: In all, since December 2010, there have been 12 cases where tapentadol was detected during routine drug screening. Eight cases had paired central and peripheral blood specimens and central/peripheral ratios averaged 1.65 and ranged from 0.54 – 3.3. The mean (median) concentration of tapentadol in central blood was 3.8 (3.3) and concentrations ranged from to <0.2 – 10mg/L. Likewise, for peripheral blood the mean (median) are 2 (2.5) and range is <0.2 – 3.1. For liver, the mean (median) and range are 10 (7.1) and <1 – 25mg/Kg, respectively. Co-intoxicants included antidepressants, antipsychotics, antihistamines, ethanol, cocaine, and miscellaneous CNS depressants.

In conclusion, of the 12 cases studied: 2 (16%) tapentadol were ruled not contributory to death, 7 (58%) were ruled accidental multiple drug intoxication, and 3 (25%) were ruled suicidal multiple drug intoxication. Concentrations of tapentadol in these groups were <0.2, 0.58 – 3.1, and 2.5 – 5.2mg/L, respectively.

Reference:

1. Winecker RE: *Quantification of Antidepressants using Gas Chromatography Mass Spectrometry*; and, *Clinical Applications of Mass Spectrometry*, Hammet-Stabler CH and Garg U, eds. Humana Press, Clifton, NJ. 2010. (pp. 45-56).

Tapentadol, Death Investigation, Toxicology

 ** Presenting Author*

K71 I'll Huff and I'll Puff: Dust Off™ Canister Abuse

Theresa M. Hippolyte, MS, Miami-Dade Medical Examiner, Number One on Bob Hope Rd, Miami, FL 33136-1133; Diane Boland, PhD, Miami-Dade Medical Exam Dept, Toxicology Laboratory, Number One on Bob Hope Rd, Miami, FL 33136; and Wilmo Andollo, BS, Dade County, Medical Examiner's Office, Number One on Bob Hope Rd, Miami, FL 33136*

After attending this presentation, attendees will be aware of the potential for abuse of Dust Off™ in combination with other drugs and the challenges of potentially misleading crime scene evidence.

This presentation will impact the forensic science community by illustrating how simple household products can be abused in conjunction with other drugs.

This presentation discusses a number of inhalant deaths that have occurred in Miami-Dade and Collier County, Florida, since 2010. The Medical Examiner's Department has investigated six cases during this time period in which the decedents were engaged in inhaling, or "huffing," 1,1-difluoroethane found in commercial products.

In addition to 1, 1-difluoroethane, the cases presented included other drugs such as benzodiazepines, opiates, anti-depressants, diphenhydramine, or synthetic cathinones and tryptamines.

The decedents were mostly Caucasian, three males and three females, ranging in age from 26 – 37 years. In all cases, the decedents were declared dead at the scene with no resuscitative efforts employed. All decedents were surrounded by multiple canisters of products containing 1,1-difluoroethane. The propellant, 1, 1-difluoroethane, typically found in products such as Dust Off™, is readily absorbed by the lungs when inhaled, and causes alcohol-like intoxication including drowsiness, lightheadedness, and loss of inhibition. At toxic levels, effects can include asphyxiation and cardiac arrhythmias. Typical users engage in the inhaling of these vapors by expelling the aerosol into a bag that is held over the head or mouth. Death can occur from either an acute episode or chronic abuse of these inhalants. The manufacturer now includes a bitterant agent in their products to deter or discourage huffing practices.

Blood samples collected during the autopsy that were used for the analysis of 1, 1-difluoroethane were stored in glass screw-top tubes. Initial volatile screening for ethanol, methanol, acetone, and isopropanol by headspace gas chromatography indicated an unknown peak on the chromatograms later identified as 1,1-difluoroethane. Follow-up confirmation was performed using solid phase micro-extraction followed by Gas Chromatography Mass Spectrometry (GC/MS). A commercial Dust Off™ product was used as reference standard for the identification of 1,1-difluoroethane. A working stock solution was prepared by spraying the aerosol into a headspace vial and capping immediately. A working control was prepared by taking a 100µL aliquot of the headspace with a gas-tight syringe from the working stock solution and infusing it into a sealed headspace vial containing internal standard (n-propanol, 15mg/L in de-ionized water). Samples were prepared by adding 1mL of blood to a vial containing 1mL of internal standard. All samples and controls were heated at 65°C for a period of 15 min prior to analysis. The analysis was performed by solid phase micro-extraction using a 75µm Carboxen-PDMS fiber (Supelco, Inc). The fiber was exposed to the samples and controls for 5 min prior to injection into the GC/MS. A 60m x 0.25mm I.D. x 1.4µm Rtx-VMS column (Restek, Inc.) was employed. Analysis was performed in the full scan electron ionization mode, with identification based on spectral library matching. The primary ions used for identification are m/z 51 (base peak), m/z 65 (major ion), and m/z 47 (major ion).

Despite the presence of multiple canisters at each scene, the cause of death is still in question. Toxicology findings in these cases indicated the decedents were abusing more than just Dust Off™. Initial interpretations based on the scene alone could be misleading without extensive toxicology follow-up. In only one of the presented cases was the cause of death solely attributed to 1,1-difluoroethane toxicity. The remaining cases are still pending the pathologist's findings due to the presence of other drugs.

Inhalant, 1, 1-Difluorethane, SPME

 ** Presenting Author*

K72 Scientific Method for Controlled Substance Analog Determination

Lindsay Reinhold, MFS, 1150 Woods Rd, Southampton, PA 18966; Heather L. Harris, MFS, JD*, 2401 Lombard St, Ste 1, Philadelphia, PA 19146; Dale E. Forrester, PhD*, USPS, National Forensic Laboratory, 22433 Randolph Dr, Dulles, VA 20104-1000; Kevin P.C. Minbiole, PhD*, Villanova Univ, 800 E Lancaster Ave, Villanova, PA 19085; Kevin G. Shanks, MS*, 2265 Executive Dr, Indianapolis, IN 46241; Jason R. Stenzel, PhD*, 580 W 7th St, Cheney, WA 99004; and J. Graham Rankin, PhD*, Marshall Univ, Forensic Science Program, 1401 Forensic Science Dr, Huntington, WV 25701*

After attending this presentation, attendees will understand relevant scientific concepts needed to comprehensively evaluate non-controlled substances as potential analogs of controlled substances, acceptance criteria associated with those concepts, the ability to establish laboratory practices to present scientific data regarding analog determination in court, and assist in the scientific prosecution or defense of analog drug cases.

This presentation will impact the forensic science community by providing laboratories with objective, science-based criteria to evaluate compounds and a means to establish consistency in analog determinations made in laboratories across the country. This presentation will introduce toxicological, chemical, and synthetic concepts surrounding the evaluation of potential controlled substance analogs and offer a scientific method for this evaluation.

The Advisory Committee for the Evaluation of Controlled Substance Analogs (ACECSA) was established by scientists from federal, state and private forensic laboratories, academia, and law to develop a scientifically valid and peer-reviewed means of evaluating the analog status of non-controlled substances and serve as a resource to law enforcement, legal counsel, laboratories, and government agencies in the scientific categorization of non-controlled substances. The Committee was gathered intentionally to maintain an independent, un-biased, and un-weighted stance in the scientific and legal communities. The main goal of constructing this group was to address a lack within the forensic chemistry field regarding the evaluation of analogs. To date, there are no guidelines, recommendations, or methods that exist in our field and no consensus or consistency in the determination of these compounds. Scientifically-sound guidelines or recommendations for analog determination are needed in the forensic arena in response to the overwhelming “designer drug” explosion and the difficult task of legislating potentially harmful new drugs.

The members of the ACECSA, in collaboration with national and international subject-matter experts, developed five aspects of a compound that should be included in evaluating analog status: Chemical Structure; Physicochemical Properties; QSAR/Computational Chemistry; Synthetic Pathway; and, Toxicology/Pharmacology will present applicable concepts and associated acceptance criteria to demonstrate the comprehensive approach to analog determination.

The Chemical Structure subcommittee aims to develop a process by which potential controlled substance analogs are evaluated and compared on the basis of their structural similarity. This structure evaluation process focuses on both 2D and 3D aspects of a chemical’s structure. Initial investigations look at core structures and functional groups.

The use of physicochemical properties in proposing potential new drug candidates has its basis primarily in the bioavailability of the compound *in vivo*. For example, solubility, partition coefficient, and pKa/pKb provide preliminary *in vitro* guidance as to the potential bioavailability.

Solubility primarily affects the dosage form and route of administration. Partition coefficient is used to predict membrane permeability. The compound’s acidity or basicity determines where an orally-administered drug might be absorbed in the body. These properties will be retrieved from scientific literature (if available) or may be calculated by modern computer programs designed and used for this purpose.

The Quantitative Structure-Activity Relationship/Computational Chemistry subcommittee will utilize available predicted activities for new chemicals. It will also evaluate the utility of similarity co-efficient models such as Tanimoto.

The goal of the Synthetic Pathway subcommittee is to analyze the structure of potential controlled substance analogs by the pathway in which they were created, i.e., deducing their chemical construction. The pathway of chemical synthesis of any organic compound can be modified by employing different building blocks; this serves as a rapid means to generate analogs of beneficial, or controlled, chemical compounds.

The Toxicology subcommittee will evaluate available pharmacological and toxicological data regarding novel compounds and compare their properties to existing controlled or scheduled drugs. This will include receptor binding and functional assay data, human and animal dosing studies, case reports, behavioral studies, adverse event reporting, and epidemiological data with clinical indicators, provided in the latter two cases that they are accompanied by analytical confirmation of the substance identity.

Analog, Controlled Substance, Method of Evaluation

 ** Presenting Author*

K73 Cross-Reactivity of Cathinone Derivatives and Other Designer Drugs in Commercial Enzyme-Linked Immunosorbent Assays

Madeleine J. Swortwood, BA, Fla Int'l Univ, 11200 SW 8th St, Miami, FL 33199; W. Lee Hearn, PhD, 425 SW Balfour Ave, Port Saint Lucie, FL 34953; and Anthony P. DeCaprio, PhD, Florida Int'l Univ, International Forensic Research Institute, 11200 SW 8th St, Miami, FL 33199*

After attending this presentation, attendees will gain an understanding of designer drugs, particularly "bath salts" or cathinone derivatives, and their prevalence in our society. In addition, the audience will also learn about the immunoassay techniques involved in screening for these substances and how the compounds cross-react in such commercial assays.

This presentation will impact the forensic science community by serving as a resource for cross-reactivity data of designer drugs in commercial Enzyme-Linked Immunosorbent Assays (ELISAs), an important factor to consider when screening biological specimens for drugs of abuse.

Designer drugs have been no stranger to the drug market in the United States over the past few decades. Recently, "legal highs" in the form of "bath salts" or "research chemicals" have dominated the drug scene as substances that are labeled "not for human consumption" in order to bypass recent regulations. While a number of bans have been put in place regarding such compounds, the abuse of these designer drugs has been on the rise while manufacturers have been staying one step ahead of the law with constantly evolving modifications to structures. When an intoxication or fatality occurs, presumptive techniques, such as immunoassays, are employed to quickly screen biological specimens for common drugs of abuse. However, since cathinone derivatives are fairly new, few assays have been created for the detection of such compounds. It is hypothesized that during routine drug screens by ELISA, the cathinone derivatives and other designer drugs may be missed. In a toxicology lab, a negative screen would not be further investigated and the substances may never be detected. For this reason, it is important to investigate the cross-reactivity of such designer drugs by analyzing across several commercial immunoassays.

In this large-scale experiment, ELISA reagents from Immunalysis, Neogen®, OraSure®, and Randox® were evaluated to determine the cross-reactivity of 30 designer drugs, including 24 phenylethylamines (including MDPV and eight cathinone derivatives), 3 piperazines, and 3 tryptamines. The study determined the percent cross-reactivity for the compounds in 16 commercial immunoassays, targeting amphetamine, methamphetamine/MDMA, benzylpiperazine, mephentermine, methylphenidate, ketamine, MDPV, mephedrone/methcathinone, PCP, and cotinine.

Cross-reactivity towards the "bath salts" was 0.5% – 4% in the assays targeting other phenylethylamines such as amphetamine or methamphetamine/MDMA. Compounds such as MDA, MDMA, ethylamphetamine, and α-methyltryptamine (AMT) demonstrated cross-reactivities in the range of 30% – 250%, but were consistent with both the manufacturer's inserts and published literature. Some assays, such as BZP, cotinine, PCP, mephentermine, methylphenidate, ketamine, and MDPV demonstrated almost no cross-reactivity toward any of the analytes evaluated. The mephedrone/methcathinone kit from Randox® demonstrated cross-reactivity toward cathinone derivatives—with false positives occurring at concentrations as low as 150ng/mL. The mephedrone/methcathinone kit was not a suitable assay for detecting other more traditional amphetamine-derived compounds but may be more fitting for screening postmortem specimens for "bath salts" when putrefactive amines may be present.

This comprehensive study determined the cross-reactivity for 30 designer drugs in biological specimens across 16 commercial immunoassay reagents. Very few "false positives" were observed in this study, which indicates the selectivity of the immunoassays and the antibodies that are employed. However, the fact that very few additional compounds were detected demonstrates a need for more broad-range screening techniques to be applied when analyzing biological specimens by immunoassays for drugs of abuse, specifically the more recent designer drugs.

Immunoassay, Designer Drugs, Cross-Reactivity

 ** Presenting Author*

K74 Analysis of Synthetic Cannabinoids Using Disposable Micropipette Extraction Tips and LC/MS

Apisri Leamniramit, BS, 1850 Park Ave, Bensalem, PA 19020; Warren Korn, BS, Center for Forensic Science Research, 2300 Stratford Ave, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to evaluate the effectiveness of disposable micropipette tips for the extraction of synthetic cannabinoids from biological samples, relative to traditional liquid/liquid extraction.

This presentation will impact the forensic science community by describing a rapid drug screening technique for a class of drugs that is becoming increasingly popular. This technique can aid in the regulation of these "legal high" products and identify their role in criminal activity or death investigations.

This project was designed to evaluate the applicability of the use of solid phase micropipette extraction tips for the isolation of synthetic cannabinoids from biological samples.

Synthetic cannabinoids are a rapidly growing class of drugs that have similar effects to those of marijuana. The scope of this class of drugs is fast-growing because their structures are easily manipulated, but can still produce cannabis-like effects. The chemical constituents of these synthetic marijuana products change frequently as attempts to regulate them evolve. Being able to detect these various drugs in biological samples has significant forensic toxicology applications. Previously, liquid/liquid extraction procedures have been performed to isolate the drugs prior to analysis by Liquid Chromatography Tandem Mass Spectrometric (LC/MS/MS) analysis; however, liquid/liquid extractions can be very time consuming, generate large amounts of waste, and involve multiple manipulations, resulting in reduced recovery.

This study describes the development of a rapid method to screen for the model synthetic cannabinoids AM-1248, AM-2201, JWH-122, JWH-210, and XLR-11using disposable micropipette extraction tips. Disposable micropipette extraction is a novel technique based on solid phase extraction. The pipette tips contain a sorbent material that binds to the sample as the solution is aspirated and expelled through the frit in the tip. These tips are advantageous over traditional liquid/liquid extraction because they are more efficient, rapid, and require lower solvent volume. Reduced solvent waste is an environmental benefit of this approach. Once extractions are performed, samples are analyzed LC/MS/MS.

Variables such as time for extraction, total solvent volume, and sample volume were evaluated as part of this assessment. A previously reported liquid/liquid extraction method was evaluated for comparison to the proposed extractions with disposable micropipette tips. The liquid/liquid extraction is performed using 1mL of sample which is acidified and extracted with chloroform/isopropanol/n-heptane, 50/17/33. Liquid/liquid extractions from serum demonstrated R^2 values above 0.98 with a linear range of 0.1 – 15 ng/mL.

Preliminary results show that the individual 10 ng/mL standards can be extracted from the micropipette tips with a significant increase in abundance when compared to liquid/liquid extractions of the 10ng/mL standards. Abundances for the micropipette tips were almost five times greater than that of liquid/liquid extractions of samples of the same concentration. Extractions with the tips also proved to be less time consuming. To prepare calibrators and perform extractions with the tips takes 2 hr as compared to 4 hr with liquid/liquid extractions. Solvent and sample volume are also decreased when utilizing the tips. The micropipette tips use 2.25mL of solvent and 0.50mL of sample volume while liquid/liquid extractions use 4.20mL of solvent and 1 – 2mL of sample volume.

Based on the work described above, disposable micropipette tips successfully extract standards of AM-1248, AM-2201, JWH-122, JWH-210, and XLR-11. The micropipette tip extraction thus far proves to have higher extraction efficiency while utilizing less sample and solvent volume.

Cannabinoids, Pipette Tip Extracts, Designer Drugs

 ** Presenting Author*

K75 Cross-Reactivity of Designer Phenethylamines With the Emit® II Plus Amphetamine/Methamphetamine Assay in Urine

Wendy R. Adams, PhD, Bill Ofsa, MS, Linay Williams, BS, and Edward J. Barbieri, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

The goal of this presentation is to provide attendees with cross-reactivity information for 4-methylethcathinone and other psychoactive phenethylamines that may aid with screening for designer drugs in postmortem and police cases. The discussion will include the parameters of the analysis as well as some specific case examples.

This presentation will impact the forensic science community by providing a fast means of screening for phenethylamine designer drugs in urine.

4-methylethcathinone (4-MEC) is a designer stimulant that has been a popular drug of abuse in New Zealand. It has been sold in the United States in bath salts products and as a research chemical. It is reportedly abused for psychoactive effects, including euphoria and alertness. 4-MEC was recently indicated in the suicide death of a New Zealand teenager and is an important compound to include in forensic toxicology testing.

NMS Labs recently reported two positive results for 4-MEC in blood that also had positive amphetamine immunoassay results in urine. None of the usual amphetamines, including amphetamine and methamphetamine, were detected in the blood using Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS). Since the structure of 4-MEC and other phenethylamines appears similar to amphetamine, it may be possible to use existing immunoassay kits to screen for this class of designer drugs.

There have been several recent reports of designer drugs producing similar positive screening results. Mephedrone was found to cross react with a methamphetamine ELISA in postmortem blood.[1] In addition, Methylenedioxypyrovalerone (MDPV) was found to cross react with PCP.[2]

In order to further characterize these potential false positive results, the cross-reactivities for several popular phenethylamines were determined by spiking standards into blank human urine. Analysis was performed using the Emit® II Plus Monoclonal Amphetamine/Methamphetamine Assay (Syva). Amphetamine, methamphetamine, cathinone, methacathinone, mephedrone, methylone, MDPV, alpha-pyrrolidinovalerophenone (alpha-PVP), 4-MEC, pentedrone, buphedrone, and naphyrone were evaluated between 500 and 10,000 ng/mL. The final concentrations in urine were verified by LC/MS/MS for those compounds with a quantitative method available. The results of this cross-reactivity testing will be presented along with some case examples.

The landscape of the designer drug market has been changing rapidly, making it difficult to develop sensitive methods for detection. Structural similarities may be used as a guide to select existing screening methods that may be sensitive to emerging designer drugs. These findings also indicate that unconfirmed methamphetamine screens could potentially contain phenethylamines. While there may be many explanations for false positive screens, the possibility of bath salt ingestion should be added to the list for consideration by medical and laboratory professionals.

This is especially important considering that one of the most attractive attributes of designer drugs is their invisibility on standard drug tests. Many analytical laboratories have been developing quantitative methods that can confirm designer phenethylamines in a variety of matrices, but targeted screening with these methods is often cost-prohibitive. The Emit® II Plus and other immunoassay tests are readily available and inexpensive tools that can potentially be validated as designer drug screens.

References:

1. Torrance H and Cooper G, The detection of mephedrone (4- methylmethcathinone) in 4 fatalities in Scotland. Forensic Sci Int 2010:202(1-3):e62-63.
2. Macher AM and Penders TM, False-positive phencyclidine immunoassay results caused by 3,4-methylenedioxypyrovalerone (MDPV). Drug Test Anal 2012: doi: 10.1002/dta.1371.

Immunoassay, Designer Drug, 4-MEC

 ** Presenting Author*

K76 Validation of Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Synthetic Cannabinoids Metabolites in Urine

Denise N. Lancaster, MS, 906 Spring St, Bristol, PA 19007; John R. Simon, PhD, Tulip Biolabs Incorporated, PO Box 334, West Point, PA 19486; and Bill Ofsa, MS, Matthew M. McMullin, MS, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to evaluate the use of immunoassay tests for the detection of synthetic cannabinoid metabolites in urine.

This presentation will impact the forensic science community by outlining an approach to method validation for immunoassay, and by providing an assessment of the merits of an immunoassay approach to the detection of these emerging compounds.

The prevalence and popularity of synthetic cannabinoid drugs has created the need for a low-cost option for screening for the presence of these drug metabolites in biological fluids. Liquid Chromatography/Mass Spectrometry (LC/MS/MS), which is in limited use as a method for screening for these compounds, requires sample extraction, lengthy run times, and is an expensive approach for high-volume screening. It also requires additional development for every new analyte or new metabolite discovered. Enzyme Linked Immunosorbent Assay (ELISA) is in widespread use for screening for many classes of drugs, and the development and evaluation of two ELISA tests to detect synthetic cannabinoids is described. ELISA is a rapid procedure that uses low-cost reagents and can be readily automated, making it an optimal technique for this process.

Following the production of antibodies in a rabbit model, antisera was harvested and antibodies isolated. After evaluating the performance of antibodies from several animals, the optimum antibody was used in a homogeneous ELISA plate immunoassay on a 96-well plate.

Two ELISA assays were developed, targeted to JWH-018 (1-naphthyl-(1-pentylindol-3-yl)methanone) and JWH-250 (2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone), respectively. At the time of development, JWH-018 and JWH-250 were the most prevalent compounds on the illicit drug market.

The assays were validated to determine their performance at the defined cut-off of 5ng/mL. The 5ng/mL cut-off was selected based on analysis of incurred authentic positive urine samples and LC/MS/MS analysis of JWH-018 and JWH-250 metabolite concentrations in authentic samples. Intraday and interday precision were evaluated by analyzing calibrators and controls over 10 days with 2 runs per day. Cut-off calibrators were run in duplicate and the mean OD used to establish the cut-off. A negative control, positive control (20ng/mL), and near cut-off concentration control (10ng/mL) were evaluated and performed acceptably for both assays under these conditions.

The assays showed significant cross reactivity with other synthetic cannabinoid standards, and metabolites. Several compounds including JWH-018 4-OH pentyl, JWH-018-5OH pentyl, JWH-081, JWH-081 4-OH pentyl, JWH-081 5-OH pentyl, JWH-122, JWH-122 5-OH pentyl, AM-2201, AM-2201 4-OH pentyl, and others generated positives on the JWH-018 assay at concentrations of less than 20ng/mL. Fewer compounds including JWH-250, JWH-250 4-OH pentyl, JWH-250 5-OH pentyl, and others generated positives on the JWH-250 assay at the same threshold. Common drugs of abuse and therapeutic drugs did not react at elevated concentrations (>10,000ng/mL).

Validation controls (positives and negatives) as determined by LC/MS/MS were presented to the ELISA methods. Subject samples testing positive for JWH-018 (5-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.1ng/mL were assessed using the JWH-018 Direct ELISA kit. There were 61 of 63 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-018 Direct ELISA kit were 96%, 100%, and 98%, respectively.

Subject samples testing positive for JWH-250 (4-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.5ng/mL were assessed using the JWH-250 Direct ELISA kit. There were 32 of 33 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-250 Direct ELISA kit were 97%, 100%, and 99%, respectively.

These ELISA assays have proven effective, sensitive, and specific for the purposes of screening for many of the currently popular synthetic cannabinoid compounds. Continued vigilance is needed to ensure that the assays will cross react with newly emerging drugs in this class.

ELISA, Cannabinoids, Designer Drugs

 ** Presenting Author*

K77 Qualitative Analysis of Designer Stimulants and Bath Salts Chemicals in Blood, Serum/Plasma, and Urine by LCTOF

Joseph Corvo, BS, Ian McGarvey, BS, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the use of Liquid Chromatography Time-Of-Flight Mass Spectrometry (LC/TOF/MS) for the screening of designer stimulants and hallucinogens in biological samples and identify considerations that reduce the risk of false positive findings.

This presentation will impact the forensic science community by increasing forensic toxicologists' understanding of the strengths and limitations of LC/TOF analysis and the need for confirmatory testing by independent methods.

LC/TOF is becoming an increasingly popular technique for drug screening in forensic and clinical toxicology laboratories. The technique is based on high-efficiency liquid chromatographic separations, coupled with a detection system that confirms the identity of the analyte by assessing its retention time and accurate mass relative to an analytical standard and its accurate mass. A panel was developed and validated for the detection of 40 analytes, including popular designer drugs such as MDPV, mephedrone, methylone, ethylone, naphyrone, 5-MeODALT, 5-MeO-DIPT, 2C-D, 2C-E, 2C-H, 2C-I, 2C-T-2, 2C-T-7,and others. The compounds were selected based on the fact that they are scheduled either at the state or federal level and are increasingly being found in "Bath Salts" type of products for purposes of abuse.

Samples (0.5mL) were buffered with 0.1M borax buffer followed by liquid-liquid extraction using 70:30 n-butylchloride:ethyl acetate. The acquisition method used a run time of 10 min with a flow of 0.700mL/min. The method used six representative deuterated internal standards to monitor analyte recovery. A target cut-off of 10ng/mL was selected for most compounds in blood and urine. Criteria used to evaluate positivity were retention time, mass accuracy, and percent concentration compared to the cutoff.

Validation of the assay consisted of assessment of precision around the cut-off, stored sample stability (room temperature (light and dark), refrigerated and frozen), carryover, autosampler stability, interference, sensitivity, and specificity.

The validation of this panel yielded an overall sensitivity of 99.7% with a range of 72% to 100% and selectivity of 99.0% with a range of 79% to 100%. Generally, analyte stability was shown to be sufficient in refrigerated and frozen samples and insufficient otherwise, whether light protected or not. Naphyrone in serum was an exception, which was shown to be unstable after one day. No carryover was detected following a sample spiked at 100 times the cutoff. Potential interferences were investigated by testing various mixed analyte pools, and did not yield any false positive findings.

The method's reproducibility was demonstrated by observing internal standard area, performance of the positive and negative controls, and the limited number of false positives. False positives only appeared to be an issue with isobaric pairs of analytes, such as 3-FMC and flephedrone, and MDMA and mephedrone. For this reason, when either member in the pair is present, confirmation testing will be done for both.

LC/TOF is a useful tool for forensic toxicology screening; however, the limitations of the technique must be acknowledged. Recognizing isobaric compounds is key to using LC/TOF as a screening tool, since the molecular mass of the analytes is the main identifying criteria. The success of the technique is highly demanding of high resolution chromatography and chromatographic quality and stability during the assay. Mass accuracy is one parameter that can help in determining the positivity of an analyte. Ion suppression due to sample matrix is another issue that must be addressed. Using representative deuterated internal standards is one way of determining the likelihood of ion suppression in a given sample.

LC/TOF, Designer Drugs, Stimulants

 ** Presenting Author*

K78 Stability of Synthetic Cathinones (Bath Salts) in Toxicology Specimens

Jeffrey P. Walterscheid, PhD, Afshin Nejabat, MS, and Michael Chen, PhD, HCIFS, 1885 Old Spanish Tr, Houston, TX 77054; and Ashraf Mozayani, PhD, PharmD, HCIFS, Texas Southern Univ, 3100 Cleburne Ave, Houston, TX 77004*

After attending this presentation, attendees will learn about the *in vitro* degradation of synthetic cathinones, often referred to as bath salts. Based on the hypothesis that beta-keto amphetamines are inherently unstable in biological matrices, a preliminary study using several cathinones spiked in blood and urine samples stored at ambient and refrigerated temperatures was evaluated by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)
and Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) over a five-week time period. This model system revealed temperature-dependent and compound-specific decay rates among the commonly known cathinones.

This presentation will impact the forensic science community by emphasizing the effects of storage conditions and type of biological sample used to perform analysis. Data reflected a rapid signal degradation, which was accelerated in ambient storage conditions and urine specimens. The values extrapolated from each experiment yielded putative rate constants, which were compared to previous actual cases with confirmed cathinones. The limited data analyzed from the five-week trial with sampling each week produced a broad generalization that could aid interpretation of results, especially when intoxication was observed yet there is an absence of confirmatory signal.

The most stable cathinone in this study was methylenedioxypyrovalerone (MDPV), which retained as much as 29% in blood stored at room temperature. In distinction, naphyrone and mephedrone showed a complete loss of response. Compared to room temperature, refrigerated blood had an overall average increase in stability by 34%. Urine samples at room temperature demonstrated the most dramatic effect between sample and temperature. All cathinones, excluding MDPV, fell sharply at day 8, followed by an even greater drop below 5% or complete loss of signal by day 29. In comparison, refrigerated urine had an overall average increase in stability by 45%. The differences show that biological specimen and temperature significantly affect synthetic cathinone stability. Both specimens produced more stability in refrigerated temperature (4°C) compared to ambient temperature (21°C). The complete or significant loss in signal occurred earliest in urine samples at ambient temperatures.

The lessons learned from this pilot study were applied to actual postmortem and DUI forensic toxicology cases. For postmortem samples collected at autopsy, the urine specimens were stored frozen at -20°C without preservative, while decomposition fluid was stored at 4°C in grey top blood tubes preserved with sodium fluoride. For DUI samples collected, the urine contained no preservative and was refrigerated at 4°C. These specimens were re-analyzed and the rate constant and half-life calculated from the stability experiment were applied and compared to previous cases. The degradation of each cathinone followed a first order rate of decay. MDPV was the compound confirmed in all cases. The rate constant determined from the pilot study agreed fairly well with actual case results.

This study indicates that it is possible for a specimen to generate a false negative result if the specimen was stored at room temperature or analyzed after a significant time delay. Refrigeration proved to lengthen stability for both types of specimens, with refrigerated urine producing the greatest stability. The stability of cathinones in biological samples is extremely important due to its increasing use among drug abusers and lack of experience in toxicology laboratories. Factors that may influence drug stability in stored samples include: storage temperature, storage time, addition of preservatives, and initial condition of the collected sample. Furthermore, LC/TOF/MS analysis revealed that as cathinone levels diminished, their corresponding “reduced” forms became more prevalent. This development may signal the need for reduced derivatives of cathinone standards for use in forensic toxicology confirmations.

Bath Salts, Stability, Toxicology

 ** Presenting Author*

K79 Forensic Investigation of PSU Herbal Incense Products Using GC/MS and LC/MS/MS

Sharyn E. Miller, BS, 1051 Teaberry Ln, Apt E109, State College, PA 16803; and Julie C. McIntosh, and Dan G. Sykes, PhD, 330 Whitmore Lab, Univeristy Park, PA 16802*

After attending this presentation, attendees will gain knowledge on herbal incense products as an emerging designer drug, applications of method development for Gas Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), in addition to the detection, identification, and quantification of synthetic cannabinoids in two commercially available products, Down2Earth Climax and Wet. In order to aid law enforcement's efforts to contain and prevent the use of these emerging designer drugs by youth, it is essential that the forensic science community develop rapid screening methods for detection that will enable rapid designer drug screening. This research will also provide significant chemical information regarding the unknown compounds in these street samples, and assist with drug awareness in a college town, a prevalent drug-consuming community, by presenting the findings at local high schools and colleges in the area.

This presentation will impact the forensic science community by developing methods for detection that will facilitate rapid designer drug screening, aiding law enforcement's efforts to contain and prevent the use of these emerging drugs.

Legal herbal products, found readily available through the internet and local gift shops, are increasingly being used for recreational drug use by youth. Marketed as herbal incense and specifically labeled "not for human consumption," these products are plant materials sprayed with cannabinoid-related chemicals that users vaporize and inhale.[1,2] Two commercial herbal samples, Wet and Down2Earth Climax, purchased in downtown State College, PA, are investigated and analyzed to determine synthetic cannabinoid presence and quantity. JWH-018, JWH-073, JWH-200, CP-47, 497, and cannabicyclohexanol are analyzed, as these represent five chemicals currently placed on Schedule I classification by the U.S Drug Enforcement Administration (DEA).[2] Scheduling was in response to an increase in the frequency of hospitalizations involving incense inhalation in the United States.[3] In addition to the five Schedule I chemicals listed above, 30 other synthetic cannabinoid related chemicals are being investigated in order to construct a library for instrumental drug screening.

To accomplish synthetic cannabinoid chemical screening, multiple extraction techniques were compared and the QuEChERS (Quick, Easy, Cheap, Rugged, and Safe) extraction method proved to be most suitable.[4] QuEChERS provides a time-effective option by combining the herbal sample with magnesium sulfate and calcium chloride buffering salts and methanol solvent in a 50mL centrifuge tube. After shaking the sample for 5 min, sample centrifuging ultimately allows for removal of the organic phase. Extracts are characterized using a GC/MS and an triple quadrupole LC/MS/MS. Optimized methods, coupled with library construction of synthetic cannabinoid standards, enable simultaneous screening of cannabinoid species, and permit a comparison of the two instrumental setups for drug screening and quantification of street herbal products.

Currently, employee drug testing does not incorporate these compounds, but as more analogs become illegal and available, screening will be forced to expand to include such chemicals. Thus, it is important to develop and validate instrumental methodology for such screening. Traditional crime laboratory drug analyses focus on GC/MS instrumentation, but semi-volatile and thermally unstable compounds may not be suitable. LC/MS/MS methods may prove more suitable for such chemicals. Preliminary results indicate a rapid, effective method for the separation and identification of synthetic cannabinoid standards and commercial herbal samples for GC/MS and LC/MS/MS. Using the method of internal calibration, deuterated analogs are utilized to quantify the synthetic cannabinoids in the samples. Products from the same brand allow for determination of inner-batch variability. Future analyses will expand to incorporate other street samples from the area, and to evaluate sample heterogeneity. Pending method optimization, the research project can be expanded to include other emerging cannabinoid-based drugs that come into vogue.

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Synthetic, Cannabinoids, Screening

 ** Presenting Author*

K80 Development of an LC/MS/MS Method for 30 Synthetic Cannabinoids and Metabolites

Erica A. Guice, MS, Matt Pentis, BS, Brad T. Holden, BS, and Mounira Ozoor, 1197 Rochester Rd, Suite K, Troy, MI 48083*

After attending this presentation, attendees will: (1) understand the challenges of testing for synthetic cannabinoids; (2) obtain knowledge on the effects of matrix on synthetic cannabinoid testing; and, (3) understand the differences in platform for synthetic cannabinoid testing

This presentation will impact the forensic science community by providing needed information for the detection of several synthetic cannabinoids that are currently being missed by laboratories.

Synthetic cannabinoids have been a topic of much discussion since their popularity as a legal high started in 2004 in Europe. To date, several states have moved to ban the use and sell of synthetic cannabinoids. However, most state and federal law makers have banned specific compounds, not structural moieties, allowing for similar compounds to come onto the market. As such, the detection of synthetic cannabinoids has always been mostly reactive.

Laboratories who generally rely on a screen-confirm methodology for their testing were further hampered until immunoassays for these compounds were developed. And, to date, most of the immunoassays developed are limited and some are already outdated. In order to remain current with the compounds on the market, based on seizures and blogs, Western Slope Laboratory developed a drug-monitoring method using liquid chromatography-online sample extraction-tandem mass spectrometry with full scan orbitrap. This method allows for targeted analysis as well as unknown compound elucidation.

With this methodology, testing is available for 30 synthetic cannabinoid compounds including metabolites. Included in the method are markers for JWH-018, JWH-073, JWH-200, JWH-019, AM-2201, JWH-122, JWH-398, JWH-022, JWH-210, JWH-015, JWH-081, JWH-020, JWH-250, HU-210, AM-694, STS-135, and XLR-11. The method tests for synthetic cannabinoids in urine and saliva.

The method is validated in concentration range of 100pg/mL – 1,000ng/mL with a Lower Limit of Detection (LLOD) below 100pg/mL and a Lower Limit of Quantification (LLOQ) at 100pg/mL. The method is linear in the aforementioned quantification range. The method was tested for matrix suppression and enhancement and none was seen in the quantification window as defined as ±25%. Imprecision has a specification limit of ±20% for all compounds; however, repeated injections (n=10) were under ±10%. Similarly, inaccuracy has a specification limit of ±20%. The method was tested to be accurate at three concentrations (low=250pg/mL, medium=50ng/mL, and high=650ng/mL) for repeated injections (n=10).

Urine samples are hydrolyzed, spiked with internal standards, and injected on the turbulent flow column. Saliva samples are spiked with internal standards, filtered, and injected onto the turbulent flow column. Standards are purchased from Cayman Chemical and Cerilliant. Mobile phase was water and methanol with ammonium formate and ammonium acetate additives. All samples were run on a Transcend TLX-2 (Thermo Scientific) coupled to a Exactive Orbitrap (Thermo Scientific). Run time for the method was under 8 min.

This method is comparable to the quantitative method previously developed at Western Slope Laboratory for synthetic cannabinoid confirmatory services. The drug-monitoring method was able to compare to the confirmatory method for the eight compounds in the confirmatory method; those compounds are JWH-200, JWH-018, JWH-018 N-pentanoic acid, JWH-073, JWH-073 N-Butanoic Acid, AM-2201, AM-694, and HU-210. The quantifiable results were similar (±10%).

In conclusion, a drug-monitoring method was developed to allow for detection of 30 synthetic cannabinoid compounds and metabolites to help in the fight against the use of these compounds. This method allows for both confirmatory testing as well as unknown identification. With this type of methodology, laboratories can now be more proactive.

Synthetic Cannabis, LC/MS/MS, Urine and Saliva

 ** Presenting Author*

K81 Designer Stimulants and Hallucinogens in Routine Casework

Sherri L. Kacinko, PhD, Wendy R. Adams, PhD, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the type and frequency of synthetic stimulants and hallucinogens seen in routine casework at a large reference laboratory.

This presentation will impact the forensic science community by providing a comprehensive review of designer drugs being seen in routine postmortem and human performance casework.

Over the past several years, the forensic toxicology community has been challenged with the emergence of large numbers of designer stimulants and hallucinogens in products commonly referred to as “Bath Salts.” The speed at which the drug-using community is able to adjust to new legislation and find compounds that are not specifically scheduled has mandated a mechanism to quickly add new compounds to the scope of routine testing. A protocol was developed to rapidly expand the Deconvolution Reporting Software (DRS) library for the Gas Chromatography/Mass Spectrometric (GC/MS) screen used in the laboratory.

Data was extracted from the laboratory information management system for all GC/MS screening analyses from January 1, 2012, to July 26, 2012. All cases in which the presence of at least one designer drug was indicated were included in the data analysis. A total of 178 such cases were identified. Pentedrone was the most prevalent compound identified (42 cases), followed by alpha-pyrrolidinopentiophenone (Alpha-PVP, 41 cases), dimethylamylamine (DMAA, 36 cases), and methylenedioxypyrovalerone (MDPV, 26 cases). Other drugs identified were methylone (16), 4-methylethcathinone (10), methylbenzylpiperazine (7), pentylone (5), butylone (5), ethylone (4), dimethylamphetamine (4), 3,4-dimethylmethcathinone (3), 1,4-dibenzylpiperazine (3), buphedrone (3), methoxetamine (2), 2C-I (2), and 1 case each of paramethoxymethamphetamine, 2C-H, 5-MeO-DALT, and paramethoxyamphetamine. Twenty-eight cases included multiple compounds. Nine of these contained didesmethylsibutramine, a metabolite of the diet drug sibutramine, but no sibutramine.

The compounds commonly found in “Bath Salts” generally fall into one of several classes of compounds, substituted phenethylamines, beta-keto cathinones, tryptamines, or piperazines. They are abused for their stimulant and/or hallucinogenic properties. The mechanism of action has not been elucidated for each compound. They generally elicit their effects by acting on serotonergic and dopaminergic receptors or by stimulating the production of related neurotransmitters. DMAA has been detected in “Bath Salts” products, specifically “PumpIt Powders,” but is not structurally similar to other common bath salt compounds. It is widely available as a supplement and used by body builders and is believed to stimulate the release of catecholamine. It does not work directly at adrenergic receptors. While 92% of cases which had a positive screen for DMAA did not appear to contain any other “Bath Salt,” three cases contained DMAA in combination with other compounds. One also contained Alpha-PVP and didesmethylsibutramine, one contained only Alpha-PVP, and one contained methylone.

The review of seven month’s worth of routine GC/MS screening data confirms the necessity of establishing a method able to detect designer stimulants and hallucinogens. These compounds are relevant to both postmortem investigations and as substances which can potentially impair driving.

Designer Drugs, Bath Salts, Postmortem

 ** Presenting Author*

K82 Postmortem Pediatric Toxicology

Robert A. Middleberg, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090; Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Ter, 850 Bryant St, San Francisco, CA 94103; Daniel S. Isenschmid, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090; Margaret Greenwald, MD*, 37 State House Station, Augusta, ME 04333; and Lucas Zarwell, MFS*, OCME, 1910 Massachusetts Ave, SE Bldg. 27, Washington, DC 20003*

After attending this presentation, attendees will gain an appreciation of the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 14th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation including pharmaco-toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause-of-death in children.

Dan Isenschmid, PhD, will be presenting a case where a 2-month infant was characterized as being “colicky.” The teenage mother, an illegal alien from Mexico, may have seen a doctor in Mexico and received medications for the infant. The infant died after administration of the obtained substances. Specimens obtained at autopsy were analyzed and determined to contain acetaminophen, metoclopramide, and chlorpheniramine, at concentrations potentially adversely affecting the health of the child. The pharmacology of these agents as well as potential toxicity to the infant will be discussed.

Peggy Greenwald, MD, will be speaking to a case involving α-PVP and pentylone in a 1-month-old infant. These relatively new compounds to the drug scene belong to the class of substances more commonly referred to as “bath salts.” Case studies involving the presence of such compounds in infants are indeed rare today. This case study will reflect on the pharmacology and toxicology of these compounds and strive to ascertain expected effects in infants.

Lucas Zarwell, MS, will discuss two cases of uncommon toxicological findings in children. The first case involves a 2-year-old that complained of “hotness.” This finding was accompanied by shaking, sweating, and gasping for air. The child became non-responsive and, despite resuscitative efforts, died. Toxicological findings demonstrated a fatal concentration of chloroquine and metabolite. Chloroquine is used in children for the treatment of malaria. As little as 1g is potentially fatal in a child. The second case involves a 6-year-old who was found in seizure. She died 2 hr later and had a negative autopsy. Toxicological analyses demonstrated a fatal concentration of the atypical antidepressant bupropion. Bupropion is used in children for such conditions as ADHD. Seizures are a minor side effect of bupropion administration in children, but have been reported.

The case studies presented reflect current-day findings in medicolegal investigations of childhood deaths. In years past, discussions of these types of cases have been educational and demonstrative of the issues in this special population. Only through these continued case studies and audience participation can there be shared perspectives on the meaning of the toxicological findings.

Pediatric, Toxicology, Postmortem

