

SOFT '96 ABSTRACTS

Society of Forensic Toxicologists

Annual Meeting

Denver, Colorado

October 14 - 18, 1996

#1 Clozapine Toxicity and Drug Interactions by Jeanne Beno*, Monroe County Medical Examiner's Office, Rochester, NY, 14623 U.S.A.

Clozapine (Clozaril) is an "atypical" antipsychotic drug particularly useful in schizophrenic patients resistant to traditional antipsychotic medications. Because there is a significant risk of agranulocytosis and seizures associated with clozapine, routine therapeutic drug monitoring (TDM) is common. We report on two fatalities in which clozapine-related drug interactions were a substantial factor in the drug's toxicity.

Case #1 was a 39 y.o. institutionalized white male on clozapine for 1 year. On 2 occasions he had seizures associated with elevated clozapine levels. He was started on phenytoin for seizure protection, but his A.M. clozapine levels plummeted and he decompensated. Due to continued behavioral problems each afternoon, his clozapine dose was steadily increased. TDM showed A.M. clozapine serum levels in the low therapeutic range. Two days after his last dose increase, he was found dead in bed.

Case #2 was a 27 y.o. institutionalized female on Clozaril for 9 months. On the maximum dose of 900 mg/day, her serum clozapine levels ranged from 0.4-0.5 mg/L. She was started on 100 mg of fluvoxamine and her clozapine levels increased 500%. Clozapine was discontinued, then gradually increased to 100 mg BID, while fluvoxamine was increased from 50 to 200 mg. She was found dead one day after her last fluvoxamine increase.

Autopsy samples were analyzed by solvent extraction and ion pair HPLC. Clozapine levels in both cases were in the toxic range. Gut and hepatic drug interactions as well as dosage scheduling may explain the toxicity.

Clozapine (mg/L)	Serum	Liver	Urine	Brain	Vitreous	Bile
Case #1	2.3	57.9	8.8	2.2	0.55	n/a
Case #2	2.8	53.4	n/a	7.6	0.35	454.0

#2 An Unusual Fatal Case of Chloroform Poisoning by Marc Deveaux*, Ahmed Semoud, Valery Hedouin and Didier Gosset, Institute de Medecine Legale, place Varlet, 59000 Lille, France and Lasir, Universite de Lille 1, 59655 Villeneuve d'Ascq, France.

Chloroform is found principally in industry and in laboratories. It does happen that people inhale vapors with the object of euphoria or as an aphrodisiac, but deaths while engaging in autoerotic activities are rare.

A case is presented of a 35 y.o. man found dead, seated in the lavatory in his laboratory, trousers down to his feet and bearing a mask filled with cotton over his face. A pornographic magazine was laying on the floor next to an empty unlabeled bottle.

Blood, urine, bile, and gastric content obtained at autopsy were submitted for alcohol analysis by GC, and for therapeutic and drugs of abuse by immunoassay. All the results were negative. A specific headspace GC on 3% SP-1500/Carbowax B procedure was applied to detect volatiles in blood. Chloroform was identified by its retention time at a low concentration of 25 mg/L.

The empty bottle was still a problem: was the chloroform coming from it? We used a gas cell dedicated for residual solvent vapor analysis with inlet/outlet ports and KBr windows. Vacuum was applied to the cell and the inlet port quickly connected to the bottle, and then closed. Fourier transform infrared analysis (FTIR) was performed directly through the cell on a Bruker IFS 48 apparatus. Chloroform was identified by its FTIR spectrum.

This case demonstrated that death is possible even with low blood chloroform concentrations. Without alcohol in the blood, one possible mechanism is that chloroform could sensitize the heart to endogenous epinephrine, which could lead to fatal arrhythmias.

#3 Tramadol Distribution in Four Postmortem Cases by Barry Levine*, Vera Ramchandar and John E. Smialek, Office of the Chief Medical Examiner, State of Maryland, 111 Penn St., Baltimore, MD, 21201 U.S.A.

Tramadol (T) is a centrally acting analgesic approved for use in the United States in 1995 for the treatment of moderate to severe pain. After a 100 mg dose, peak plasma concentrations are about 0.3 mg/L; peak plasma steady state concentrations are about 0.6 mg/L. Over the past year, T has been identified in 4 cases investigated by the Office of the Chief Medical Examiner, State of Maryland. None of these deaths were attributed to T intoxication. T is an alkaline extractable drug and elutes from a DB-1 column with good chromatographic characteristics without derivatization. In all cases, T was confirmed by full scan electron impact gas chromatography-mass spectrometry. Quantitative results are provided below:

Specimen	Concentration (mg/L or mg/Kg)			
	Case 1	Case 2	Case 3	Case 4
Heart blood	4.4	1.4	1.8	0.17
Peripheral blood	6.5	1.0	1.7	0.27
Urine	210	29	26	30
Bile	11	0.75	1.9	0.31
Liver	7.0	1.5	2.6	0.30
Kidney	13	1.6	2.3	0.43

In all cases, concentrations of T were at least an order of magnitude higher in urine than in blood or other tissues or fluids. The liver and kidney do not appear to be major storage depots for the drug.

#4 Death Following Intentional Methyl Bromide Intoxication by Aristidis M. Tsatsakis* and Maria G. Christakis-Hampsas, Toxicology Unit, University of Crete, Iraklion GR 71409; Manolis N. Michalodimitrakis and Nikolaos Trikilis, Forensic Pathology, University of Crete, Iraklion GR 71409; Panagiotis Christodoulou, Dept. of Otolaryngology, University of Crete, Iraklion GR 71409.

A case of a death due to methyl bromide (MeBr) is presented. In previously reported fatalities due to MeBr inhalation, only inorganic bromide levels in biological specimens were reported. Intact methyl bromide has so far not been isolated from human tissues following exposure. In the present case, MeBr was detected, confirmed, and quantitated in biological samples by headspace gas chromatography, using a modified technique of our previously reported methodology for freon-22 determination. The victim was found unresponsive soon after exposure and was immediately transferred to the University Hospital. Post mortem toxicology revealed MeBr in peripheral blood (3.3 mg/L) and in subclavian blood (3.8 mg/L). Lung, brain, gland, kidney, liver, and testis levels of MeBr were as follows: 3.9, 3.5, 3.4, 2.6, 1.9, and 2.8 mg/g, respectively. MeBr was also detected in bile (1.2 mg/mL). No other drugs or volatile compounds were detected in the autopsy specimens. Inorganic MeBr concentrations in the aforementioned biological samples were also quantitated. A slightly different distribution of inorganic bromide in tissues from that observed for methyl bromide was shown; inorganic bromide in blood, 530 mg/mL, brain, 38 mg/g, kidney, 310 mg/g. The cause of death was ruled as MeBr poisoning; the manner suicide.

#5 Detection of Cocaine and Its Metabolites Using the PharmCheck™ Sweat Patch by Maxine Warren, Neil Fortner, Robert Fogerson and Jacquie Suttiff, PharmChem Laboratories, Menlo Park, CA U.S.A.; Hilary Liberty, National Development and Research Institutes, New York U.S.A.

The purpose of this study was to develop methodologies and testing techniques for sweat patch collection and laboratory analysis, to compare and evaluate the agreement between the urine EMIT cocaine assay and a microplate ELISA cocaine assay for sweat patches, and document the reliability and validity of sweat patches for detecting cocaine and its metabolites. In conjunction with National Development and Research Institutes, 27 self reporting cocaine users were studied over a 16 day period in East Harlem, New York. Urine samples and sweat patches were obtained from these individuals over the 16 day period. All urine specimens were screened using the EMIT cocaine assay with a 300 ng/mL cutoff. All sweat patches were screened using a microplate ELISA cocaine assay with a cutoff of 10 ng/mL. All positive screening results were confirmed using GC/MS. Comparison of the cocaine urine and patch screening data showed a 93% correlation. Comparison of the urine EMIT cocaine data with the sweat patch GC/MS data showed a 89% correlation. Comparison of the GC/MS data for positive sweat patches and positive urine samples showed a 81% correlation. As a commercial product, the sweat patch (PharmCheck™) offers several advantages to urine testing. It is a non-invasive sampling technique, provides a longer period of detection for monitoring drug use, is difficult to adulterate or beat, and provides a deterrent effect via continuous monitoring of the individual.

#6 Sweat Testing for Heroin and Metabolites in a Heroin Maintenance Program by Pascal Kintz* and Patrice Mangin, Institut de Médecine Légale, 11 rue Humann, 67000 Strasbourg, France; Rudolf Brenneisen and Petra Bundeli, Institute of Pharmacy, Baltzerstrasse 5, 3012 Bern, Switzerland.

For many years, toxicologists have detected the presence of drugs of abuse in biological materials using blood or urine. In recent years, remarkable advances in sensitive analytical techniques have enabled the analysis of drugs in unconventional specimens such as sweat. In a study conducted during a heroin maintenance program, sweat patches were applied to 14 subjects. They were administered heroin in 2 or 3 intravenous dosages/day. Heroin dosages ranged from 80 to 1000 mg/day. The sweat patch was applied 10 min. before the 1st dose and removed about 24 hours later, some minutes before the 3rd or 4th dose. The absorbent pad was stored in plastic tubes at -20°C until analysis. The target drugs were extracted in 5 mL acetonitrile in the presence of 100 ng of heroin-d₃, 6-monoacetylmorphine-d₃, and morphine-d₃. After 30 min. agitation, the acetonitrile solution was divided into 2 portions: 2 mL for heroin testing and the remainder for the other compounds. After evaporation, the first residue was reconstituted in 35 mL acetonitrile and the second was derivatized by silylation with 40 mL BSTFA + 1% TMCS. Drugs were analyzed by GC/MS in electron impact mode. Concentrations ranged from 2.1 to 96.3, 0 to 24.6, and 0 to 11.2 ng/patch for heroin, 6-MAM, and morphine. No correlation between the doses of heroin administered and the concentration of heroin in sweat was observed.

#7 Fetal Cocaine Exposure: Analysis Of Vernix Caseosa by Christine Moore* and Douglas Lewis, U.S. Drug Testing Laboratories, 2201 W. Campbell Park Drive, Chicago, IL U.S.A.; Delia Dempsey, San Francisco Bay Area Regional Poison Control Center and Dept. of Pediatrics, San Francisco General Hospital, University of California (UCSF), 1001 Potrero Avenue, San Francisco, CA U.S.A.

Preliminary data regarding the use of vernix caseosa (VC) as an alternative to other biological specimens for the determination of fetal cocaine exposure are presented. Advantages of VC analysis include its presence on all newborn babies, historical record of drug exposure, and ease of collection and storage. Newborns are released from hospital care within hours of birth, so many are discharged before meconium can be collected for drug testing. Neonatal urine is difficult to collect, and not all newborns have hair. Vernix caseosa is present on the skin of all newborns in differing amounts depending on the degree of prematurity (more premature babies have more VC). Fifteen (15) samples of vernix caseosa — five from babies known to be cocaine exposed because of a positive benzoylcegonine result from the urine and umbilical cord blood; ten from non-exposed neonates — were analyzed for the presence of cocaine and metabolites using solid-phase extraction (Isolute HXC; Jones Chromatography, Denver, CO) and gas chromatography-mass spectrometry (GC/MS). VC samples from three of the five neonates known to be cocaine exposed were positive for cocaine and/or its metabolites, the other two had little or no remaining specimen. The remaining ten were negative. More research is required into the diagnostic validity of this alternative sample matrix for the determination of fetal drug exposure.

#8 Identification of Unique Cocaine Analytes In Meconium of Cocaine Exposed Fetuses by Jonathan M. Oyler*, P. Suess, W.D. Darwin and E.J. Cone, Addiction Research Center, DIR, NIDA, NIH, Baltimore, MD U.S.A.

Confirmation of fetal cocaine (COC) exposure can have significant medical and forensic repercussions. Recently, meconium analysis for COC and its analytes has been found to be a useful method for detecting fetal drug exposure. We studied the disposition of COC in meconium specimens from six COC-exposed neonates. Mothers were positive for prepartum COC use by urinalysis and/or self-report. Specimens were analyzed for COC and 12 related analytes by solid phase extraction followed by gas chromatography/mass spectrometry. Analytes were identified in meconium by comparison with authentic standards. The previously identified analytes COC, ecgonine methyl ester, cocaethylene (CE), benzoylecgonine (BZE), norcocaine, benzoynorcocaine, and m-hydroxybenzoylecgonine (m-HOBZE) were detected. Additionally, the following new analytes were identified in meconium: anhydroecgonine methyl ester (AEME), ecgonine ethyl ester (EEE), norcocaethylene (NCE), m- and p-hydroxycocaine, and p-hydroxybenzoylecgonine (p-HOBZE). AEME was present in all specimens in concentrations from 12-178 ng/g. CE and EEE were detected in four specimens, three of which also contained NCE. Five of the six specimens contained p-HOBZE in concentrations ranging from 15-709 ng/g. In addition, the ratio of p-HOBZE/m-HOBZE was >1 in two of the samples. We also investigated the crossreactivity of COC analytes in the TDx and EMIT immunoassays for COC metabolite. Only BZE, m-HOBZE and p-HOBZE demonstrated high crossreactivity (>100 %). Identification of these unique COC metabolites in meconium suggested the following: since AEME is a pyrolysis product, its presence in meconium demonstrated placental transfer directly from the mother during gestation; identification of cocaethylene and the products of cocaethylene metabolism, EEE and NCE, confirmed fetal ethanol exposure during the last two trimesters of pregnancy; and the high prevalence of p-HOBZE indicated that it may also be a valuable marker of fetal COC exposure. In addition, the high crossreactivity of both m- and p-HOBZE in the EMIT and TDx assays for COC metabolite suggests that confirmation of COC metabolite should include BZE, m-HOBZE, and p-HOBZE.

#9 A Comparison Between Phenobarbital and Phencyclidine Binding in Pigmented Hair by Matthew H. Slawson*, Michael Chamberlain, Diana G. Wilkins and Douglas E. Rollins, Center for Human Toxicology, Dept. of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT U.S.A.

The ionization state of a drug may be an important factor in the binding and retention of drugs in hair. Melanin is a highly anionic polymer that results in the pigmentation of hair. Drugs which retain a positive charge at physiological pH may be expected to be more highly retained in hair than a drug that is neutral or negatively charged at physiological pH due to the ionic interactions between the cationic drug and anionic melanin. It can be hypothesized that by altering the extent of ionization of drugs incorporated into hair, they may be selectively removed from hair based on the percentage of nonionized drug formed at a given pH. To test this hypothesis, phencyclidine (PCP) was chosen as a model cationic drug and phenobarbital (PB) was chosen as a model anionic drug. Male Long-Evans rats were injected ip with either PCP or PB at 10 mg/kg/day or 40 mg/kg/day for 5 days. Fourteen days later, pigmented hair was collected and pooled. The two groups of hair were extracted daily for 10 days with 200 mM phosphate buffer at pH 6.5, 8.5, or 10.5 for the PCP (pKa = 8.5) group, and 5.4, 7.4 or 9.4 for the PB (pKa = 7.4) group. Hair was then analyzed for the retained PCP or PB. The PCP group showed a pH dependent decrease in the amount of drug remaining in hair when compared to nonextracted hair, from 64% Key Words: Hair, Drug Incorporation, Ionization State.

#10 Characterization of Cocaine Binding Sites in Hair by Robert Joseph*, Tsung-Ping Su and Edward J. Cone, ARC, NIDA, NIH, Baltimore, MD 21224 U.S.A.

In vitro studies have demonstrated that reversible, saturable, and stereoselective cocaine binding sites exist in hair. We further evaluated binding sites in hair by determining the *in vitro* effects of pH, NaCl, and temperature on [³H]cocaine binding to six light and dark colored hair specimens. Suspensions that contained hair in 50 mM Tris-HCl buffer (pH 7.4) were prepared to measure total binding with 500 nM [³H]cocaine followed by incubation at 25°C for 1 h prior to filtration. Hair suspensions that contained [³H]cocaine also were prepared at pH 3.0, at pH 10.5, with 0.49 M NaCl, or with 4.9 M NaCl. In other assays, hair suspensions were incubated at 4°C or 80°C. In all assays, nonspecific binding was defined by 10 mM cocaine. Specific binding represented the difference between total and nonspecific binding. The preliminary results are listed in the following table (*denotes a significant (p<0.05) difference compared to control):

Measure	0.49 M NaCl (% of control ± S.E.M.)	4.9 M NaCl (% of control ± S.E.M.)	pH 3.0 (% of control ± S.E.M.)	pH 10.5 (% of control ± S.E.M.)	4° C (% of control ± S.E.M.)	80° C (% of control ± S.E.M.)
Non-specific	55 ± 9*	61 ± 9*	51 ± 9*	36 ± 8*	88 ± 7	103 ± 10
Specific	53 ± 10*	19 ± 7*	37 ± 10*	14 ± 7*	102 ± 22	106 ± 37

These findings indicate that NaCl affects [³H]cocaine binding in hair. [³H]cocaine binding was also affected by suspension pH which may have been due to changes in the ionization state of [³H]cocaine and functional groups that comprise binding sites.

#11 In Vitro Cocaine Metabolism in Plasma: Influence of Butyrylcholinesterase by Rebecca A. Jufer*, Jonathan M. Oyler, Gilberto N. Carmona and Edward J. Cone, Addiction Research Center, DIR, NIDA, Baltimore, MD U.S.A.; Nigel H. Greig, GRC, NIA, NIH, Baltimore, MD U.S.A.

Cocaine is metabolized by plasma esterases to ecgonine methyl ester via enzymatic hydrolysis. Butyrylcholinesterase (BChE) appears to be the enzyme responsible for this conversion. *In vitro* studies were conducted to investigate the role of BChE in cocaine metabolism and esterase activity in several animal species. Rhesus monkey plasma was supplemented with horse serum BChE (100 units) and samples were spiked with cocaine. Aliquots

were removed at specified timepoints following incubation at 37°C for four hours. Cocaine and analytes were analyzed by solid-phase extraction followed by gas chromatography/mass spectrometry in the SIM mode. Limits of detection for cocaine, ecgonine methyl ester and benzoylecgonine were approximately 1 ng/mL. The half-life of cocaine in plasma supplemented with BChE was 7.5 min. compared to 37.7 min. for plasma containing only endogenous BChE.

In a second *in vitro* experiment, the relative cocaine metabolizing ability of pooled rhesus monkey plasma versus squirrel monkey plasma was assessed. Cocaine was added to each plasma type, and samples were incubated at 37°C for 1 hour. Aliquots were removed at designated timepoints and samples were analyzed for cocaine and analytes by GC/MS. Cocaine elimination half-lives were 20.1 min. and 60.2 min. for rhesus monkey plasma and squirrel monkey plasma, respectively. The decreased cocaine elimination half-life in rhesus plasma was attributed to higher endogenous BChE activity in rhesus monkeys.

These data demonstrated the importance of BChE as a metabolic pathway for cocaine biotransformation. The increased rate of elimination of cocaine from plasma suggested that BChE administration could serve as a treatment for cocaine overdose.

#12 Determination of Tetrahydrocannabinol - Accuracy, Precision, Limit of Detection and Limit of Determination as found by 4 Collaborative Studies and Proficiency Testing in the FRG by Rolf E. Aderjan* and Michael Herbold, Institute of Legal Medicine, University of Heidelberg, Voßstr. 2, D - 69115 Heidelberg; Manfred R. Möller, 2 Institute of Legal Medicine, University of Saarland, Homburg; Herbert Käferstein, Institute of Legal Medicine, University of Köln; Gerold Kauert, Institute of Legal Medicine, University of Frankfurt; Thomas Daldrop, Institute of Legal Medicine, University of Düsseldorf.

By a new legal provision being introduced by the German federal government in 1996, the presence of abused drugs in blood during driving in a concentration above an analytical threshold will be regarded as a violation. Three rounds of reference value determination and round robin testing (designed according to the "Harmonised protocol of the AOAC/ISO/IUPAC") for four targeted drugs/drug metabolites have taken place up to now. The participants (n= 57-42) were asked to determine tetrahydrocannabinol (THC), morphine, benzoylecgonine and amphetamine in lyophilised serum samples using their own practically employed method.

Validation data for GC/MS determination of THC (SIM and deuterated internal standard) and interlaboratory comparison are especially important as in practical case work for the determination of this analyte often has to be carried out near the limit of detection. Accuracy and precision at three concentration levels during proficiency testing and an additional collaborative study of 11 experienced labs each analyzing 10 of 110 randomly distributed lyophilised serum samples (spiked with 0, 0.5, 1.0, 1.5, 2.0, and 4.0 µg/L THC) show, that the practical limit of determination is approximately 5 µg/L serum and the limit of detection is 2 µg/L serum, when a correct identification of THC is taken into account. THC concentration, accuracy, and precision data will be presented using graphs.

#13 A Four Year Evaluation of Treatment and Outcomes After Reported *Loxosceles reclusa* Envenomation by Kari Blaho*, Mike Cockrell, Stephen Winbery and Kevin Merigian, Department of Emergency Medicine and Clinical Toxicology, UT Memphis, TN 38163 U.S.A.; Stephen Geraci, Department of Medicine, UT Memphis, TN 38163 U.S.A.

Loxosceles reclusa (brown recluse spider) is commonly found in the midsouth region of the U.S. The characteristic lesion produced by the brown recluse is usually detected by the patient hours or days after envenomation. Death is an uncommon sequela. There are currently no widely available laboratory tests to accurately diagnose envenomation in an acute care setting. Diagnosis is based on identification of the spider and acute clinical findings. We report the results of a 4 year retrospective study on reported brown recluse spider bites in an inner city medical center. The study was prompted by an ongoing controversy in the role of certain drugs as therapeutic interventions. We report the clinical findings, treatment regimen and outcome of 224 patients who presented with spider or insect bites in a 4 year period. Of these, 87% were discharged home, 12% were admitted to a floor bed, 0.8% were admitted to an ICU. There was only one death during this time. The mean age of the patient population was 33 years, and the majority of patients were female. Average vital signs at the time of presentation were within normal limits. Several therapeutic agents were used for the treatment of brown recluse bites, most were not consistently effective. Of the 224 patients in this study, the most common treatments rendered were systemic antibiotics, antihistamines, and nonsteroidal agents. Results from this study indicate that documented brown recluse bites are relatively uncommon, as are deaths due to brown recluse envenomation.

#15 An Abuscreen® OnLine Immunoassay for the Detection of LSD in Urine on the Cobas Integra Clinical Analyzer by Krystyna Goc-Szcutnicka, Maria G. Bates, Sharmila Honasoge, Alan J. McNally* and Salvatore J. Salamone, Roche Diagnostic Systems, Inc., Somerville, NJ 08876

We have developed an Abuscreen® OnLine Immunoassay for the detection of LSD in human urine on a new clinical analyzer, the Cobas Integra. The Cobas Integra has the capability of fully automating a variety of homogenous assays for the detection of electrolytes, abused drugs, chemistries, specific proteins, and therapeutic drugs. This homogenous immunoassay for LSD is based on the Kinetic Interaction of Microparticles in a Solution (KIMS). Drug content in a urine sample is directly proportional to the inhibition of microparticle aggregation. The assay cutoff is 0.5 ng/mL and the clinical sensitivity for the detection of LSD and its metabolites in human urine is equivalent to the LSD Abuscreen® RIA. Test results are reported in a qualitative format, with absorbance values equal to or less than the cutoff, considered positive.

The performance of the OnLine LSD reagents on the Cobas Integra was evaluated using the following criteria: precision, clinical performance, correlation to GC/MS/MS, assay specificity (848 presumptive negative samples), cross-reactivity, and limit of detection (LOD). The intra (n=20) and inter

(n=100) assay precision had %CVs values of $\leq 4.25\%$ at 0.25 ng/mL, 0.5 ng/mL, and 0.75 ng/mL. Twenty eight GC/MS/MS positive samples were analyzed on the Cobas Integra. All samples were positive. Eight hundred and forty eight presumptive negatives were also analyzed on the Integra; 845 were negative and 3 were positive (0.35% false positive rate). Cross-reactivity studies showed a greater than 35% cross-reactivity to nor-LSD and 2.5% to iso-LSD. The LOD was found to be 0.064 ng/mL.

In summary, the Abuscreen[®] LSD OnLine homogeneous, non-isotopic immunoassay on the Cobas Integra meets the sensitivity requirements for the detection of LSD and its metabolites in human urine.

#16 GC/MS Procedure for the Analysis of Flunitrazepam and Its Metabolites in Urine by Mahmoud A. ElSohly* and Shixia Feng, ElSohly Laboratories, Incorporated, 5 Industrial Park Drive, Oxford, MS 38655 U.S.A.; Salvatore J. Salamone and Robert Wu, Roche Diagnostic Systems, Somerville, NJ 08876 U.S.A.; Rudolf Brenneisen, University of Bern, Bern, Switzerland.

Analysis of urine specimens collected from individuals ingesting 2 and 4 mg flunitrazepam (FN) showed positive results by OnLine and OnTrak immunoassays for up to 60 hours. GC/MS analysis of these specimens for FN, 3-OH-FN, 7-amino-FN, 7-amino-3-OH-FN, desmethyl-FN, and 3-OH-desmethyl-FN after glucuronidase treatment showed only low levels of 7-amino-FN with almost none of the other metabolites. These levels were far below expected results based on the immunoassay data. This study reports on a GC/MS procedure for FN and the above metabolites and for the 7-amino-desmethyl-FN and its 3-OH-derivative. The method is based on acid hydrolysis of the urine specimens which converts FN and all its metabolites described above to one of four amino-benzophenone derivatives (1-4), with d₅-oxazepam as internal standard. Under the experimental conditions, the internal standard is converted to 2-amino-5-chloro-d₅-benzophenone. The LoD for all compounds tested was 5 ng/mL. Analysis of urine specimens collected for 72 hours post ingestion of 1, 2, or 4 mg FN showed appreciable levels of benzophenone-3 (product of 7-amino-FN and 7-amino-3-OH-FN) and lower levels of benzophenone-4 (product of 7-amino-desmethyl-FN and 7-amino-3-OH-desmethyl-FN), with no detectable levels of benzophenones-1 and -2. The method makes it possible to confirm the presence of FN metabolites in urine at least 72 hours post ingestion of small doses of the drug.

#17 Direct Measurement of Testosterone and Epitestosterone Glucuronides and Sulfates by HPLC/MS/MS by Larry D. Bowers* and Sanaullah, Athletic Drug Testing and Toxicology Laboratory, Indiana University Medical School, 635 Barnhill Drive, Indianapolis, IN 46202-5120 U.S.A.

Performance enhancement in athletics through the use of drugs has been a controversial issue since the original Olympic games in Greece. The use of endogenous steroids, such as testosterone and dihydrotestosterone, has presented a particular problem for athletic drug testing. The testosterone/epitestosterone ratio (T/E) has been used to assess exogenous testosterone administration; a ratio greater than 6 being interpreted as a positive result. The routine testing procedure with GC/MS has been criticized for measuring only the glucuronide forms of T and E. Steroids are normally excreted in urine as conjugates with glucuronide, sulfate, and perhaps other compounds.

We have developed a direct HPLC/MS/MS method for quantitative analysis of testosterone and epitestosterone glucuronide and sulfate. The urine sample was adjusted to pH 4.5, applied to a Varian C-18 solid phase cartridge, and eluted with 50/50 methanol/water. The residue was injected into an isocratic (45/55 MeOH/10 mM NH₄CH₃COO) reversed phase HPLC system and all of the effluent directed through an Ionspray interface to an API III^{Plus} MS/MS system. Deuterated conjugates, synthesized in our laboratory, were used as the internal standards for each corresponding sulfate or glucuronide. Interface and collision-induced dissociation conditions strongly influence the observed fragmentation; sulfates and glucuronides showed very different behavior. Selected reaction monitoring (SRM) was used for quantitation.

The HPLC/MS/MS technique has been used to analyze urine specimens from athletes and volunteers. In 6 of 18 males, the inclusion of the sulfate conjugates significantly lowered the T/E ratio, although none of the subjects would have been misclassified. We found low concentrations of testosterone glucuronide in 7 of 10 Chinese men, while testosterone sulfate concentrations were normal.

#18 Comparison of Flunitrazepam Excretion Patterns Using the Abuscreen OnTrak and OnLine Immunoassays and GC/MS by Salvatore J. Salamone*, Cheryl Brenner, Sharmila Honasoge, Alan J. McNally, Joseph Passarelli, Krystyna Szkutnicka, Roche Diagnostic Systems, Somerville, NJ 08876 U.S.A.; Rudolf Brenneisen, Univ. of Bern, Bern, Switzerland; Mahmoud A. ElSohly and S. Feng, ElSohly Laboratories, Oxford, MS U.S.A.

A study was conducted to compare the performance of the OnLine and OnTrak immunoassays for benzodiazepines with gas chromatographic/mass spectrometric (GC/MS) analysis in detecting flunitrazepam (FNP) and its metabolites in human urine. Urine was collected over a 72-hour period from seven individuals (4 male, 3 female) who had taken a single oral dose of either 1, 2, or 4 mg of FNP. The OnTrak assay was run at a 100 ng/mL cutoff with nordiazepam (NDP), and the OnLine assay was run with a standard curve from zero to 200 ng/mL of NDP with and without beta-glucuronidase treatment. Each sample was analyzed by GC/MS using FNP, and 7-amino-FNP (7-AFNP) as standards with beta-glucuronidase treatment. The specimens from the 1 mg dose did not yield a positive result by immunoassay over the 72 h collection period. Specimens from the 2 and 4 mg doses did yield positive results in both immunoassays. The time of the first positive results ranged from 4 to 12 h and the time to the last positive result ranged from 18 to 60 h. Treatment of the samples with beta-glucuronidase increased the OnLine values between 20 and 60% but did not appreciably increase the detection time. GC/MS analysis showed no detectable levels of FNP; however, all samples collected past time zero showed detectable levels of 7-AFNP (>2 ng/mL) with peak concentrations at 12-36 h. The peak levels of 7-AFNP by GC/MS paralleled the peak levels of the immunoassay response. Further studies to identify additional metabolites by GC/MS are in progress.

#19 A Method for the Detection of Cocaine and its Metabolites and Various Opiate Drugs in Blood Utilizing Solid-Phase Extraction by Gary W. Davis* and Kenji Ota, California Department of Justice Toxicology Laboratory, Sacramento, CA U.S.A.; Daniel E. Coleman, Chemical Toxicology Institute, Foster City, CA U.S.A.

This laboratory is frequently requested to analyze various drugs of abuse in blood samples. Due to the low levels of drugs and their metabolites that are encountered in blood samples, very sensitive extraction procedures are required. We have developed a simple method for the detection of cocaine, benzoylecgonine, and various opiate drugs such as morphine, codeine, and 6-monoacetylmorphine that utilizes solid-phase extraction columns combined with GC/MS and/or GC/MS/MS to successfully detect low levels of these compounds. These drugs can be, if necessary, analyzed simultaneously from the same one mL aliquot of the blood sample.

The blood sample containing the appropriate deuterated internal standard (i.e. morphine- d_3) was first precipitated using acetonitrile. After centrifugation, the solution was acidified and put through a simple clean-up step. The sample was then prepared for solid-phase extraction and placed on a SPEC[®]MP3 microcolumn. The resulting eluant was evaporated and derivatized with BSTFA with TMCS. The extract was then analyzed using either GC/MS or GC/MS/MS. The instrumental analysis was done using both electron impact ionization (opiate drugs) and positive chemical ionization (cocaine and benzoylecgonine). For GC/MS analysis, one ion per compound was used to detect the parent compound and its deuterated internal standard (i.e. m/z 429 for morphine and m/z 432 for morphine- d_3) except in the case of codeine where two ions each were used for both the drug and the internal standard. When GC/MS/MS was selected, the drugs were detected by selecting one appropriate parent to daughter transition that were specific for the analyte of interest and its deuterated internal standard (i.e. m/z 304 to 182 for cocaine and 307 to 185 for cocaine- d_3).

The assay is linear from 1 to at least 100 ng/mL. When GC/MS/MS is used all the above drugs have a limit of detection of 1 ng/mL and a limit of quantitation of 5 ng/mL. We have developed a rapid and sensitive method for the detection of cocaine and opiate drugs in blood samples that is able to detect low levels of drugs using a minimal sample volume. In addition, the solid-phase extraction procedure minimizes the use of toxic extraction solvents and mitigates the associated costs of solvent use.

#20 The Alkylation of Drugs at Ion Exchange Sites on the Surface of Solid-Phase Extraction (SPE) Columns by Kathy A. Rymut, Ed T. Heebner, Zheng Lu, Glynn Chaney and Michael J. Telepchak*, United Chemical Technologies, Inc., 2731 Bartram Road Bristol, PA 19007 U.S.A.

Ion exchange extraction columns have been widely used for the extraction of various drugs. Studies have shown that ion exchange columns prepared in different ways could cause conversion of drugs to form alkyl derivatives during extraction procedures. The alkyl derivatives that are formed can cause drug confirmation results to be reported as false negatives.

The purpose of this paper is to study the alkylation mechanism to minimize or eliminate formation of alkyl derivatives during the extraction processes. Comparison studies of alkylation were carried out by passing each drug through the solid-phase extraction (SPE) column. The drugs tested include norcodeine, norcocaine, normorphine, nordiazepam, and benzoylecgonine. Urine samples were spiked and extracted along with unextracted methanolic standards, using the low cut-off ranges specified by SAMSHA guidelines for GC/MS confirmation i.e., 300 ng/mL for norcodeine and 150 ng/mL for benzoylecgonine. Conversion of these compounds to their alkyl derivatives were analyzed by GC/MS in the SIM mode. Among the three preparation conditions, sorbents A & C showed 28 and 21% conversion respectively of norcodeine to codeine; 59 and 13% conversion of norcocaine to cocaine; and 44 and 10.5% conversion of normorphine to morphine. Benzoylecgonine and nordiazepam were unaffected at levels less than 200 ng/mL, although benzoylecgonine does show significant conversion at higher concentrations. No alkylation was observed for the drugs in sorbent B. In addition, we have compared this sorbent with other commercially available products which demonstrate 8% conversion of norcodeine to codeine and 11% conversion of normorphine to morphine. The data suggests that the hydroxy group has a higher alkylation ability when compared with acid and amide which are unreactive under the same conditions.

The conclusion of this study shows that alkylation can be a major factor for inaccuracies when reporting final drug testing results, especially at the lower concentrations which can lead to false negatives. To prevent this, manufacturing techniques have been developed to eliminate alkylation and the possibilities of false negatives.

#21 A Procedure for the Identification and Quantitation of Fenproporex by Sandra Valtier* and John T. Cody, Clinical Investigations, Wilford Hall Medical Center, Lackland AFB, TX 78236-5319 U.S.A.

Drugs that are metabolized to amphetamine or methamphetamine are potentially significant concerns in the interpretation of amphetamine positive drug tests. One of these compounds, fenproporex, is an anorectic drug that is available in many countries. The fact that it is metabolized to amphetamine has already been established in the literature. Following administration, the parent compound has been reported to be detectable only for several hours while amphetamine can be detected for days. Because of complications with interpretation of amphetamine positive drug tests, the viability of a current amphetamine procedure was evaluated for identification and quantitation of this compound.

Ions acquired for fenproporex were m/z 293, 240, 118, 91, and 56. All 5 ions were evaluated to assess the best 3 ions to select for routine monitoring. Use of the 240 ion exhibited interference with some urine samples particularly at low concentrations and therefore was quickly rejected as a candidate. The other ions, however, proved to be viable alternatives. HP-1 and DB-17 capillary columns were used to ensure elimination of interference from related amines. Internal standards amphetamine- d_6 and methamphetamine- d_{11} were evaluated in this study for the quantitation of fenproporex. The limit of detection was determined to be 2 ng/mL, the limit of quantitation was 5 ng/mL, and the linear range was shown to be 5 to 5,000 ng/mL. All characteristics (variability, accuracy, etc.) were determined using both internal standards and a determination made regarding the utility of each. Precision

studies showed deuterated methamphetamine as internal standard provided the most consistent results (within-run CV of 4.98%, and between-run CV of 12.5%) compared to deuterated amphetamine (within-run CV of 11.13%, and between-run CV of 18.36%).

#22 - A Rapid High-Pressure Liquid Chromatography Assay for the Determination of Lysergic Acid Diethylamide (LSD) in Blood by *Jana L. Champion* and Kristin L. Rogahn, Wisconsin State Crime Laboratory-Milwaukee, 1578 South 11th Street, Milwaukee, WI 53204-2860 U.S.A.*

Illicit use of lysergic acid diethylamide (LSD) reached peak popularity in the mid-1960s and subsequently declined in the 1970s. Presently, its popularity is regional but is increasing in some focalities such as the midwestern United States. The combination of reversed-phase high-performance liquid chromatography (HPLC) and enzyme immunoassays (EIA) were used for a rapid detection of LSD in whole blood. The assay employs a commercially available STC-LSD micro-plate EtA kit and a liquid-liquid extract. Chromatography was performed with a Metachem Inertsil ODS2 (250 x 4.6-mm i.d.) reversed-phase column, fluorescence detection at 254 nm, and methanol-phosphate buffer (90:10, v/v) as mobile phase. Linear standard curves were obtained over the concentration ranges of 1.0 to 10 ng/mL in whole blood. The inter- and intra-day coefficients of variation for the assay were <20% at low concentrations (1-4 ng/mL) and <10% at high concentrations (5-10 ng/mL).

#23 Glycerin Presence Causing False Positive Methanol By Gas Chromatography by *George F. DuPre*, C. Andrew Robinson, Jr.* and Marilyn Hall, Div. Forensic Pathology/Dept Pathology, University of Alabama at Birmingham, WP P230, 618 S. 18th Street, Birmingham, AL 35233 U.S.A.*

An alcoholic patient entered the emergency room (ER) during the weekend with vomiting, seizures, and hyponatremia. It was thought by the ER physician that the patient had consumed antifreeze. A request for an alcohol screen and ethylene glycol was placed. When this case presented, the lab was not fully staffed. The lab reported a methanol of 154 mg/dL. Treatment began with an ethanol drip. An astute resident, who had rotated through forensic toxicology laboratory overheard the lab results. It was known that a volatile in unopened headspace tubes causes a false positive methanol. Routinely, the lab performs the test using a Monoject sterile blood collection tube in which the stopper was removed for several days. Alcohols are determined using headspace analysis by gas chromatography. The test was performed using a tube in which the stopper had not been removed. The test was repeated with a properly prepared tube and the false positive methanol was no longer present. The manufacturer states the tubes contain glycerin.

A study was undertaken to see if glycerin caused the positive methanol result. Upon analysis of a 0.1% solution of glycerin, the GC indicated a methanol concentration of 41.3 mg/dL. A calibration curve was constructed for methanol. Next, the effect of the amount of time the stoppers were removed from the tube versus the false positive methanol concentration was graphed to determine how long the stoppers should be removed to allow the "methanol-like" compound to dissipate. An unopened tube resulted in a false positive for methanol of 177.7 mg/dL.

It is our recommendation to open red top tubes for at least 24 hours when these tubes are used for headspace analysis.

#24 GC/MS Confirmation of Barbiturates by *Robert C. Meatherall*, Laboratory Medicine, St. Boniface General Hospital, 409 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6*

A GC/MS method was developed to simultaneously identify and quantitate the most commonly used barbiturates in blood and urine specimens. The 6 targeted barbiturates were butalbital, amobarbital, pentobarbital, secobarbital, mephobarbital, and phenobarbital. Tolybarbital (p-methylphenobarbital) was used as the internal standard.

Following liquid-liquid extraction and solvent evaporation, the residue was dissolved in acetonitrile and alkylated with ethyliodide using tetramethyl-ammonium hydroxide as the catalyst. The resulting N-ethylated barbiturates were well resolved from each other on a non-polar methylsilicone capillary column. Day-to-day precision resulted in CVs between 5% and 9% between 200 ng/mL and 5,000 ng/mL for both blood- and urine-based standards. The method was linear from 50 to 10,000 ng/mL.

#25 The Confirmation of Benzodiazepines in DUI cases by GC/MS by *H. Chip Walls* and Bernard W. Steele, University of Miami, School of Medicine-Forensic Toxicology Laboratory, Miami, FL U.S.A.; Richard Maclure and W. Lee Hearn, Toxicology Laboratory, Dade County Medical Examiners Office, Miami, FL U.S.A.*

This paper describes extraction procedures and analytical methods for the confirmation of urinary benzodiazepines. The following drugs and their metabolites are targeted: alprazolam, bromazepam, chlordiazepoxide, clonazepam, diazepam, flunitrazepam, flurazepam, lorazepam, nordiazepam, oxazepam, temazepam, and triazolam. Initial screening of urine samples is by immunoassay (OnLine®).

After enzymatic hydrolysis (b-glucuronidase type IX-A; E. coli), the free benzodiazepines are recovered from alkalized urine by liquid-liquid extraction using hexane:methylene chloride mixed solvent. The organic solvent is evaporated to dryness. The residue is solubilized in acetonitrile and then derivatized with MTBSTFA. Sample preparation time averaged 6 hours. A GC-MS selected ion monitoring acquisition method targeting retention time, selected ion abundances, and qualifier ion ratios was used to determine positive results. The recovery of most drugs and metabolites exceeded 90%. Exceptions were recoveries for alpha-hydroxyalprazolam, alpha-hydroxytriazolam, and lorazepam which were greater than 66%. Linearity was demonstrated from 0.1 to 1.0 mg/mL for each drug. Within-run CVs were less than 11%, and between-run CVs were less than 16%.

The most common benzodiazepines detected in Dade County DUI cases using this technique were alprazolam/metabolites, diazepam/metabolites, and flunitrazepam metabolite. The analytical results from 50 DUI cases demonstrate the primary urinary metabolite of flunitrazepam to be 7-aminoflunitrazepam ranging in concentration from 35 to over 2,000 ng/mL.

#26 Identification and Quantitation of Free Opiate Compounds in Blood and Urine by Ion Trap GC/MS/MS and GC/MS³ by Cheryl A. Ehorn*, Varian Analytical Instruments, 3050 Hansen Way, M/S V-130, Bldg. 5A, Palo Alto, CA 94304 U.S.A.; Dale R. Somers, Maureen Black and Roger Tatum, Bio-Tox Laboratories, Division of U.S. Alcohol Testing Inc., 10410 Trademark Street, Rancho Cucamonga, CA 91730 U.S.A.

Analytical specificity for free opiates in blood and urine is difficult due to matrix interferences, poor extraction efficiency, low therapeutic levels, and poor chromatographic behavior. A simple procedure for the rapid, sensitive, and specific identification and quantitation of opiates was developed using Ion Trap GC/MS/MS and GC/MS³. Splitless injection into a 15 m Rtx-5-Amine column was used for all separations. Electron ionization (EI; 70 eV) was initially employed for all the opiates using the most abundant, high mass ion as the parent/precursor. Positive chemical ionization (CI, acetonitrile, 42u reagent ion) demonstrated enhanced signal-to-noise (x 10) for hydrocodone, dihydrocodeine, and codeine using the respective M+1 ions as the parent/precursor. Energy for the collision induced dissociation (CID) was applied as either non-resonant or resonant excitation waveforms. The simpler, non-resonant method was used for all MS/MS dissociations, while the more selective, resonant method was applied in both MS³ dissociation steps. LOQ was less than 1 ng/mL for all the opiate and opiate metabolites included in this study. Stable ion ratios (\bar{n} 10%) were maintained across the linear range (at least three orders of magnitude) for all analytes. RSDs will be reported for 10 replicates at 1 pg on column (extract of 1 ng/mL with an average recovery of >90%).

#27 Analysis of Methadone and Its N-Demethylation Metabolites by GC/PICI-MS: Applications for Human Plasma, Urine and In Vitro Metabolism by David E. Moody*, Mario E. Alburges, Wei Huang and Rodger L. Foltz, Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112 U.S.A.

Methadone is often invoked for detoxification and maintenance of the opioid addict. We have developed and validated a sensitive and specific method for the analysis of methadone and its metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP), in human plasma, urine, and liver microsomes. This assay employs a solid-phase extraction. Separation and analysis of the analytes are performed by capillary gas chromatography (GC)/positive ion chemical-ionization (PICI)-mass spectrometry (MS). The protonated ions (MH⁺) monitored are m/z 264 and 267 for EMDP-d₀ and -d₃; m/z 278 and 281 for EDDP-d₀ and -d₃; and m/z 310 and 313 for methadone-d₀ and -d₃. Recovery of methadone and its metabolites exceeded 85%. Standard curves were linear from 10-600 ng/mL (average r²: methadone-0.997; EMDP-0.990 and EDDP-0.998; n = 3). With plasma fortified at 25, 100, and 300 ng/mL, the assay was precise (intraassay CVs 4-9%; interassay CVs 1-13%) and accurate (intraassay % target 86-106%; interassay % target 88-98%) for all 3 analytes. Similar validation criteria were met in human urine and liver microsomes. Methadone and its metabolites were stable in plasma and urine at room temperature for at least one week and in microsomes for at least 24 hours. In 22 plasma specimens, where methadone ranged from 33 to 358 ng/mL, EDDP was detected in the 15 specimens with higher methadone concentrations (methadone to EDDP ratios of 5.6 to 15.1). EMDP was not detected. In 5 urine specimens where methadone concentrations ranged from 126 to 3873 ng/mL, EDDP was consistently detected (methadone to EDDP ratios of 0.04 to 0.71). Low concentrations of EMDP (32-33 ng/mL) were detected in 3 of the urine specimens. Incubation of methadone with human liver microsomes resulted in a time and substrate concentration-dependent production of EDDP. EMDP production was not detected. Incubations with P450-selective inhibitors and cDNA expressed P450s suggest that methadone N-demethylation is mediated by P450 3A4. (Supported by contract N01DA1-9205)

#28 Simultaneous Analysis of Opiates in Urine by SPE and GC/MS with Stabilization of Keto-Opiates via Conversion to Oxime Derivative by Clifton W. Jones*, DVA Medical Center, Bay Pines, FL 33504 U.S.A.; Glynn Chaney, United Chemical Technologies, Inc., Bristol, PA 19007 U.S.A.; Stephen Mastorides, USF College of Medicine, Tampa, FL 33617 U.S.A.

Synthetic opiates including hydromorphone (HM), hydrocodone (HC), and oxycodone (OXYC) can account for a large number of positive opiate immunoassay results. Concurrent use of these drugs with codeine (COD), morphine (MOR), and/or heroin is not uncommon. Simultaneous quantitation of opiates and their synthetic analogs by GC/MS can be problematic due to similar retention times and mass ion profiles. Enolization of keto groups in the synthetic opiates hinders accurate quantitation and can cause interference in the identification and quantitation of codeine and morphine. We demonstrated a rapid assay to simultaneously quantitate total MOR, COD, HM, HC, and OXYC utilizing acid hydrolysis, solid-phase extraction (SPE), and GC/MS. Enhanced separation and stabilization of keto opiates was facilitated by conversion of keto functional groups to an oxime derivative prior to extraction. Analytes were subsequently converted to trimethylsilyl (TMS) derivatives post extraction.

Glucuronide conjugates are cleaved by adding 0.5 mL concentrated HCl to each 5 mL urine sample. Samples were hydrolyzed for 15 minutes in a boiling water bath. After pH adjustment, 0.2 mL of 10% hydroxylamine (HOX) was added to convert keto opiates to their corresponding oxime form. CLEAN SCREENTM SPE columns (200 mg) were used for extraction. Extraction conditions were as follows: Add 1 mL phosphate buffer (0.1M, pH 6.0) to the hydrolyzed sample, vortex, and centrifuge. The columns were conditioned by sequentially passing 3 mL methanol, 3 mL DI water, and 1 mL phosphate buffer. After the sample was applied to column, the columns were washed with 2 mL DI water, 2 mL sodium acetate buffer (0.1 M, pH 4.5), and 3 mL methanol. The analytes were eluted with 3 mL methylene chloride/isopropanol/ammonium hydroxide (78/20/2). The extract was evaporated to dryness, derivatized with 50 mL MSTFA and 25 mL ethyl acetate, and heated at 80°C for 15 minutes. The derivatized extract was assayed by GC/MS (EI) operated in the SIM mode.

Standard curves for all five compounds were linear (r²>0.995) over the concentration range of 125-1000 ng/mL. Analytical recoveries exceeded 85% for all analytes. Limit of detection (LOD) was 65 ng/mL or less for all analytes.

The procedure describes a dual derivative mechanism for improved separation and quantitation of opiates and their keto isomers. Sample preparation and extraction is rapid, efficient, and clean. Results show good correlation and sensitivity.

#29 MTBSTFA Reduces Phentermine Interference in the GC-MSD Analysis of Amphetamine by Michael S. Cremese*, Edward F. O'Connor, and Alan H.B. Wu, Toxicology Laboratory, Hartford Hospital, 80 Seymour St., Hartford, CT 06102

Phentermine (PHE) is an anorectic agent that can be a problem interferent in GC-MS analysis of sympathomimetics, particularly amphetamine (AMP). While resistant to elimination by periodate oxidation, PHE interference can be reduced by approaches which increase chromatographic resolution of PHE from AMP. Some interference remains if the PHE concentration is very high. MTBSTFA (N-methyl-N-(tert-butyl-dimethylsilyl)trifluoroacetamide, Pierce) has been used as a derivatization agent to increase resolution, but the methodology is complex. This complexity derives from the requirement for quantitative yields for methamphetamine (MAMP) and PHE, while AMP derivatizes with MTBSTFA quantitatively without additional steps. We found that simple derivatization of samples with MTBSTFA reduces PHE interference substantially. This approach also reduced the yield of MAMP.

We demonstrated the utility of this approach as part of a two step process. In the first step, urine samples positive for AMP were treated with periodate, extracted using the Toxi-Lab A system, derivatized with PFPFA (penta-fluoropropionic acid anhydride, Pierce), and analyzed using a HP U-2, 33 micron, 12m x 0.22 mm column in an HP 5890 GC interfaced to an HP 5970 MSD. If PHE was absent, both AMP and MAMP were quantitated. When PHE was present and interfered with AMP quantitation, only MAMP was measured. In order to quantitate AMP, a second aliquot was extracted and derivatized with MTBSTFA. This results in a reduction of the yield of PHE and eliminates interference.

#30 Ion Trap MS/MS Detection Methods for Drugs of Abuse by Kathryn S. Kalasinsky*, Teresa Schaefer and Joseph Addison, Office of the Armed Forces Medical Examiner, Armed Forces Institute of Pathology, Division of Forensic Toxicology, Washington, D.C. 20306 U.S.A.

This study concentrates on developing mass spectrometry (MS) methods for detection of drugs of abuse from urine for the traditional "NIDA 5" drug classes (cocaine, opiates, amphetamines, cannabinoids, and phencyclidine) and their metabolites utilizing ion trap MS/MS techniques. The goal of this study was to find methods which could greatly improve the limits of detection and reliability by eliminating matrix interference. Tandem MS/MS spectrometers have the advantage of background elimination by transmitting MS output of a select ion range to a second MS for analysis. The ion trap MS/MS has an advantage over the quadrupole MS/MS in costs and ease of analysis. Operational parameters for the drug analyses in electron impact (EI) mode in MS and MS/MS were determined on the ion trap instrument. Positive chemical ionization (CI) MS/MS was also explored and typically greater detection limits compared to EI were attainable depending on the compound. The instrumental methods were evaluated at 10%, 1%, and 0.1% of the Department of Defense (DoD) confirmatory cutoff limits for a positive sample. In all cases, good quality data were obtainable at or below 0.1% of the current cutoff value. The full scan data available from the EI/MS method of the ion trap aids in drug confirmation when combined with the EI/MS/MS and CI/MS/MS. Detection limits were generally 0.1 ng/ml or better. Comparisons of sample analyses with other techniques show the ion trap MS/MS to be superior to quadrupole EI/MS in the select ion

#31 Today's GC/MS Drug Testing Laboratory: Automation of Extraction, GC/MS Instruments and Data Review by Michael A. Peat and Alan E. Davis*, LabOne Inc., Overland Park, KS U.S.A.; Nick Bukowski, Finnigan Corporation, Manchester, UK.

Historically, confirmations in drug testing laboratories have been performed by solid-phase extraction (SPE) and GC/MS in the selected ion monitoring mode. The development of regulatory programs has led to the introduction of strict quality control and data review criteria, and because of the absence of integrated systems, manual intervention has been necessary in assaying a batch of specimens by GC/MS and in the review of the GC/MS data.

This presentation describes the operation of an integrated process involving a robotic system for dilutions and extractions, and GC/MS instrumentation capable of enhancing production efficiency. Because of the sensitivity characteristics of the Finnigan MD 800, extracts can be assayed in the split mode allowing for rapid injection cycles. In addition, software development allows the instrument to perform real-time data review; for example, injections that fail one of several pre-established chromatographic parameters are automatically re-injected. After the batch has been injected, it is electronically reviewed using pre-established quality control rules. No manual intervention is necessary during either the injection process or the post-injection batch review process. These enhancements have led to a significant improvement in productivity.

#32 GC/MS Values for THCA in DHHS Regulated Workplace Specimens by Elizabeth S. Keith*, William R. Lynn, Barbara Rowland and John Irving, Laboratory Corporation of America, Memphis, TN U.S.A.; William B. Keith, University of Mississippi, University, MS U.S.A.

Urine specimens from DHHS regulated clients were used: (1) to determine the GC/MS concentration distribution of THCA (11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid) in workplace specimens screened at 100 ng/mL versus those screened at 50 ng/mL; (2) to determine if the drug testing program could further be enhanced by lowering the GC-MS cutoff concentration to 10 ng/mL; and (3) to relate the above compilation of THCA concentrations to the need to retest positive specimens after frozen storage. Another objective was to obtain data to answer the most frequent question from commercial clients, "What is a high drug concentration?"

In July and August 1994, specimens (n=32,367) screened at 100 ng/mL cutoff yielded 459 positive for cannabinoids; 419 (91.3 %) of these had a GC-MS level of > 60 ng/mL, and only 40 (8.7 %) had levels of 20-60 ng/mL. During September and October 1994, specimens (n=31,885) screened at 50 ng/mL cutoff resulted in 648 positive for cannabinoids; 375 (57.8%) had a GC-MS level > 60 ng/mL, 263 (40.6%) had levels of 20-60 ng/mL, and 3

specimens contained <15 ng/mL THCA. Positive THCA specimens (GC-MS >15 ng/mL) increased from 1.42 % to 2.1% with the 50 ng/mL screening cutoff. In September and October 1995, similar results were obtained from specimens (n=34,100) screened at 50 ng/mL cutoff. Even with 50% loss from storage, THCA levels should be sufficient for retests. Lowering the GC/MS cutoff to 10 ng/mL would not significantly increase the number of positive specimens.

#33 Separation and Measurement of DimethylPolysiloxanes by Gas Chromatography/ Atomic Emission and Mass Spectrometric Detections by Subbarao V. Kala*, Ernest D. Lykissa and Russell M. Lebovitz, Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 U.S.A.

Though the implantation of Polysiloxanes has been used in cosmetic and prosthetic medicine for a long time, there has been a lack of a unequivocal methodology for monitoring the leaching of polysiloxanes in the recipient's body.

We have developed a method for the detection and quantitation of polysiloxanes in tissues using the HP Pascal GC/AED system equipped with a J&W Scientific DB-1HT 30 m, 0.32 mm ID, chromatographic column and a HP 6890/5972 GC/MS equipped with a similar column of 0.20 mm ID. In the case of the GC/AED system, the silicon atomic emission was monitored at 251.514 nm. The GC/MS was operated both in the scan mode for initial detection and SIM modes for enhanced sensitivity. The injector inlets of both the instruments were operated in the splitless mode and were set at 300°C. Oven temperature program was set initially at 80°C and ramped 20°C/min to a final temperature of 350°C.

Various concentrations of dimethylpolysiloxanes (9-913 ng/mL) (DMPS-V, d=0.913 g/mL, Sigma) were used to generate calibration curves with GC/AED and GC/MS systems. The linear regression coefficients for the twelve peaks identified in DMPS-V ranged from 0.96 to 0.99. The silicon content for each of these peaks was quantified using octaphenylcyclotetrasiloxane (EM Science, 1 g Si in 7.06 g) standard. Since dimethylpolysiloxanes contain 37.8% silicon, the amount of DMPS in each peak was recalculated from their silicon content. The total content of DMPS-V used for generating a chromatogram was accounted by the twelve identifiable peaks. The detection limit for the measurement of dimethylpolysiloxanes for GC/AED and GC/MS were 50 and 10 pg, respectively. Studies with extraction of liver homogenates spiked with DMPS-V showed that 10 min extraction using ethyl acetate was adequate to recover 98% of added silicone. To examine the non specific binding of these dimethylpolysiloxanes to tissues homogenates, various amounts of DMPS-V (9 to 91 mg) were added to 200 ml of liver homogenates (10% in 25 mM Tris-HCl, pH 7.0) and were incubated at room temperature for various periods of time (1, 24 and 72 hr) and were extracted in 1 ml of ethyl acetate for ten minutes. The recovery of DMPS-V (98%) was found to be unaffected with the increasing incubation time. Thus, the present methodology enables us to achieve high sensitivity for the detection of silicones in biological tissues.

#34 Analysis of Paracetamol Using Solid-Phase Extraction, Deuterated Internal Standards, and Gas Chromatography-Mass Spectrometry by David J. Speed, Stuart J. Dickson, Eric R. Cairns and Diana C. Kappatos*, ESR, P.O. Box 30547, Lower Hutt, New Zealand.

Paracetamol (acetaminophen) is one of the most used analgesic drugs and is often implicated in forensic cases. Forensic casework requires a high degree of specificity as well as sensitivity, precision, and linearity over a wide range of concentrations.

The method employs solid-phase extraction (Blood Elut Certify I cartridges), a deuterated internal standard, and butylation derivatives using butyl iodide and TMAH (2.5% in methanol). Gas chromatography/mass spectrometry offers the specificity required.

The intraday and interday variation was obtained for paracetamol in blood, ranging from 5 -200 mg/mL (sub-therapeutic to mid-fatal), and was as follows:

Intraday (n=10); CV 3 -6%

Interday (n=8); CV 4 -20%

The linearity obtained for a five-point calibration curve in blood from 2.5 -280 mg/mL was $r^2 = 1.000$. The method has been automated using the Zymark Rapid Tracer.

#35 New Findings on Hydrolysis of Cannabinoid Conjugates by Imad K. Abukhalaf^{1,3}, Dempsey D. Alford^{1,3}, Barbara R. Manno^{2,3}, Joseph E. Manno^{1,2,3} and Philip M. Kemp⁴, ¹Departments of Medicine and ²Psychiatry, and the ³Center of Excellence for Clinical and Forensic Toxicology, Louisiana State University Medical Center, Shreveport, LA, and ⁴Chief Medical Examiner's Office, Oklahoma City, OK U.S.A.

Cannabinoids excreted as conjugates require hydrolysis prior to analysis. Kemp *et al.* (JAT 19:285-291; 19:292-298) reported that the method of hydrolysis can influence cannabinoid(s) concentration in specimens and showed quantitative differences between hydrolysis using alkaline and b-glucuronidase methods. We now describe utilization of alkaline phosphatase and sulfatase enzymes for hydrolysis of cannabinoid-sulfur and cannabinoid-phosphoester conjugates in human urine. Urine specimens collected from five human subjects approximately two to three hours after each had smoked a marijuana cigarette (3.54% THC) were pooled. Urine aliquots (1 mL) were subjected to one of the following hydrolysis treatments: b-glucuronidase from *Escherichia coli* (bacteria), b-glucuronidase from *Helix pomatia* (mollusks), b-glucuronidase from *Patella vulgata* (limpets), sulfatase from *Helix pomatia*, alkaline phosphatase from bovine intestinal mucosa, acid and alkaline hydrolysis, and no hydrolysis. Specimens were incubated, extracted, and derivatized for gas chromatographic-mass spectrometric analysis. Cannabinoids of interest were: cannabinol, cannabidiol, D⁹-tetrahydrocannabinol (THC), 11-nor-THC-9-carboxylic acid (THCCOOH), 11-hydroxy-THC, 8a-hydroxy-THC, 8b-hydroxy-THC (8b-OH-THC), and 8a,11-dihydroxy-THC. Low concentrations of 8b-OH-THC were detected only with extraction at neutral pH; THC was detected only with *E. coli* b-glucuronidase; THCCOOH was detected with alkaline phosphatase but in decreased amounts; and other cannabinoids were detected with sulfatase or b-glucuronidase hydrolysis. (Supported in part by NIDA grant DA-05850; LSUMC-S IRB approved)

#36 Fatty Acid Ethyl Esters in Maternal and Fetal Rat Tissues After Ethanol Ingestion by Guy M. Vallaro, George S. Behonick, John N.D. Wurpel and Jesse H. Bidanset*, St. John's University, Jamaica, NY 11439 U.S.A.; C. Nicholas Hodnett, Westchester Dept of Labs & Research, Valhalla, NY 10595 U.S.A.

Fatty acid ethyl esters (FAEEs), non-oxidative metabolites of ethanol, are found in human and animal tissues. Study objectives were to identify and quantitate palmitic and stearic FAEEs in maternal and fetal tissue after acute ethanol ingestion. Four timed pregnant Long Evans rats were assigned to one of three groups (2 g/kg ETOH as 25% w/v p.o.). Blood specimens were collected prior to euthanasia 0.5, 1.5 & 3.0 hrs post dose. Maternal brains and livers were recovered at necropsy along with combined fetuses. Blood ethanol concentrations were determined by a Perkin-Elmer Sigma 2000 headspace GC (n-propanol internal standard). For brain, liver, and fetal FAEE concentration, tissues were homogenized, extracted in toluene:hexane (1:1) at pH-5 (ethyl heptadecanoate internal standard), and evaporated to dryness under nitrogen. Residues were injected into a Hewlett-Packard GC/MSD with a HP Innowax GC-column. The instrument, operated in the SIM mode, monitored ions 88, 101, and 157. Blood ethanol concentrations 0.08 and 0.05 g% were determined for the 0.5 and 1.5 hr groups. No alcohol was found in blood from the 3.0 hr group. Palmitic FAEE and stearic FAEE were detected in maternal blood, brain & liver for all 3 time points. Both FAEEs were detected in pooled fetal tissue while positive controls exhibited mean values of 72.67 (SEM 7.17) and 10.67 (SEM 1.15) ng/g stearic FAEE and palmitic FAEE, respectively.

Time (hr)	Fetal palmitic FAEE (ng/g ± SEM)	Fetal stearic FAEE (ng/g ± SEM)
0.5	201.5 ± 9.5 *	272.5 ± 6.5 *
1.5	12.0 ± 12.4 *	179.0 ± 29.9 * = p<0.05
3.0	35.7 ± 20.7	90.3 ± 38.95

FAEEs are in fetal tissue and maternal blood, brain, and liver after acute ethanol. FAEE deposition in tissues may be a possible biochemical marker for Fetal Alcohol Syndrome.

#37 Effects of Sodium Fluoride-Thymol and Temperature on the *de novo* Production of Ethanol in Urine over Time by Robert D. Williams*, Christopher R. Gibson and Nicole S. Blumenshein, Ohio State University Medical Center, 1214 Kinnear Road, Columbus, OH 43212 U.S.A.

The effects of sodium fluoride-thymol (SFT) on the *de novo* production of ethanol in human urine were studied over time. Forty-five specimens were randomly chosen from 23 male and 22 female Medical Center patients and subjected to a screen for ethanol using the Syva enzyme assay technique. Positive specimens were confirmed and quantitated using GC-FID. Each specimen was divided into three separate ten mL aliquots which were either pipetted directly into a plastic urine container, added to a container with one SFT tablet (10.2 mg NaF), or supplemented with 0.2 g/dL ethanol. The aliquots were retained in these original containers at room temperature (23°C) and assayed for ethanol at various times over a period of one month. One specimen, obtained from a female produced ethanol over the time course in the presence of 1 mg/mL sodium fluoride (0, 0.355, 1.590 g/dL ethanol) and its absence (0, 0.445, 1.583 g/dL) at 0, 4, and 30 days, respectively. This specimen possessed a high concentration of glucose and upon culturing, was found to contain *Enterococcus Faecalis*, *Klebsiella Oxytoca*, *Pantoea Agglomerans*, and *Candida Albicans*. Based on this sampling, it is concluded that the synthesis of substantial ethanol concentrations *de novo* can occur in a small percentage of urine specimens in the presence or absence of 1 mg/mL sodium fluoride when the specimens are maintained at room temperature for an extended period of time. Therefore, higher concentrations of sodium fluoride (1%) are recommended to reduce the possibility of microbial fermentation and ethanol production [Lough and Fehn, J. Forensic Sci. 1993; 38(2):266-71].

#38 DWI Forensic Blood and Urine Alcohol Quality Control Program in Minnesota by Subhash G. Jejurkar and Glenn G. Hardin*, MN BCA FS Lab, 1246 University Ave., St. Paul, MN 55104 U.S.A.

The aspects of this program that are monitored include the sample collection kits, ethanol analyses, reporting, and courtroom testimony.

One percent of the blood and urine sample collection kits made to our specifications are selected at random and analyzed for the presence of sodium fluoride (NaF) and potassium oxalate using ion chromatography techniques. Blood collection tubes must contain between 80-120 mg NaF (potassium oxalate must also be present), and urine collection kits must contain between 800-1200 mg NaF, before collection kits from those manufacturing lots can be released for field use.

Ethanol analyses were performed using headspace gas chromatography with five calibrators ranging from 0.05-0.30 g/dL for instrument calibration. Seven controls ranging from 0.025-0.40 g/dL are analyzed in duplicate with each batch of samples. All control results must be within ±3.0 percent of their expected values, except the 0.025 control which must be within ±0.003 g/dL of its expected value.

Every evidentiary sample is analyzed on two separate days. The replicate results must be within ±3.0 percent of each other.

#39 Compatibility Study of LSD and its Analogs Using Various Solvents and Storage Conditions by Mitzi M. McDowell* and H. Kenan Yaser, Radian International LLC, P.O. Box 201088, Austin, TX, 78720-1088 U.S.A.

The development of new methods for the analysis of lysergic acid diethylamide (LSD) and its analogs has created a need for high-quality, stable analytical reference standards. A study was conducted to evaluate the stability of these materials in solution. The compounds included in this study were LSD, LSD-D₃, and iso-LSD. Each of the materials was synthesized, and the identity and purity were verified using multiple analytical methods. Standard solutions were prepared in acetonitrile, methanol, and 1,2-dimethoxyethane (DME) and stored at various temperatures. A method was developed to

analyze the standards using high performance liquid chromatography (HPLC) with UV detection. The method specifications included using a Partisil ODS-5 column (4.5 x 250 mm); acetonitrile/0.01 M potassium phosphate buffer (80/20) mobile phase, flow rate of 1.5 mL/min, and wavelength of 268 nm. Baseline analyses at T₀ were performed on all solutions. The test solutions were analyzed periodically over a three-month period by comparison to an internal standard. Preliminary analysis of standards prepared in the three solvents indicate no significant differences in standard concentration (<5%). At the end of this period, the neat materials were monitored for degradation. Although no degradation was observed by HPLC, some discoloration of the neat material had occurred. Stability data was obtained by comparison of the freshly-prepared standard in acetonitrile to an existing standard in acetonitrile. This analysis resulted in a % difference of <5%. The selected solvent and storage combination for long-term stability for each standard is acetonitrile stored under refrigeration.

#40 Pharmacokinetics of Ibogaine and Its o-Demethylated Metabolite Noribogaine in Rats and Monkeys by John P. Pablo*, Lionel P. Raymon and Deborah C. Mash, Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL 33136 U.S.A.; William L. Hearn, Metro-Dade County Medical Examiner's Dept., Number One on Bob Hope Road, Miami, FL U.S.A. 33136-1133; Michael H. Baumann, Clinical Psychopharmacology Section, National Institute on Drug Abuse/NIH Addiction Research Center, Baltimore, MD 21224 U.S.A.

Ibogaine, an indole alkaloid found in the root of an African rainforest shrub *Tabernaemontana iboga*, is currently being evaluated for its potential as an addiction interrupting agent. The purpose of this study was to determine the pharmacokinetic parameters of ibogaine and its metabolite noribogaine following administration of ibogaine (at various doses) either orally (p.o.) or intravenously (i.v.) to rats and monkeys. Analyses were performed by published methods (Hearn et al., *JAT* 19:427-434, 1995). Nonparametric analysis of monkey (*Macaca fascicularis*) whole blood time-concentration profiles of ibogaine from i.v. (10 mg/kg) administration resulted in terminal half-life values (avg. \pm SEM) of 2.06 ± 0.47 , and 2.35 ± 0.33 hr. for the male and female respectively. The clearance rate from blood was 41.0 ± 0.1 , and 30.0 ± 6.0 ml/min/kg for the male and female respectively. Bioavailability was calculated from the nonparametric constants obtained from experiments using various oral doses (25, 50, and 100 mg/kg). Oral bioavailability of ibogaine was less than 10% for either male or female.

Pharmacokinetic analysis of ibogaine and noribogaine in specific brain regions of rats (Sprague Dawley) orally administered ibogaine (50 mg/kg) demonstrated elevated levels (1-5 mg/kg tissue) for ibogaine as early as 15 minutes post-dosing. Similar concentrations of noribogaine were first evident after 1 hour in agreement with first-pass metabolism of the parent drug. In addition, brain regional profiles were different for the parent drug and metabolite. In contrast to the elevated levels in brain, systemic concentrations (whole blood) of the rat analytes were similar (0.20-0.70 mg/L) to the whole blood values from identically dosed monkeys. The return to baseline levels for both analytes within 12 hours in whole blood, combined with the high brain to whole blood ratios of these compounds, suggests that ibogaine and its metabolite may become trapped within the CNS compartment.

#41 Quantitative Determination of LAAM and Its Major Metabolites DinorLAAM, and NorLAAM in Hair by PCI-GC/MS by Angelique S. Valdez*, Diana G. Wilkins, Steven P. Gygi and Douglas E. Rollins, The Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah U.S.A.

A highly sensitive method has been developed for the quantitative determination of l-a-acetylmethadol (LAAM) and its metabolites in hair. LAAM, l-a-acetyldinormethadol (dinorLAAM), and l-a-acetylnormethadol (norLAAM) were spiked onto 20-mg samples of human hair. Deuterated internal standards of LAAM, dinorLAAM, and norLAAM were added at 20 ng/mg. Calibration standards containing known concentrations of LAAM, dinorLAAM, and norLAAM dried onto human hair were also prepared. Samples were digested overnight in Protease K (2 mL) at 37°C in a shaking waterbath. The pH of all samples was adjusted to 9.0 with 50% w/v potassium phosphate dibasic buffer. N-butyl chloride (2 mL) was then added and samples extracted for thirty minutes followed by centrifugation to achieve separation of organic and aqueous layers. The organic phase was evaporated to dryness at room temperature and reconstituted in 30 μ L of n-butyl chloride. Analysis was performed by positive chemical ionization GC/MS on a Finnigan MAT 4500 mass spectrometer coupled to a 9610 gas chromatograph. Methane and ammonia were used as reagent gases. The assay was linear from 0.1 ng/mg (norLAAM) or 0.3 ng/mg (LAAM, dinorLAAM) to 50 ng/mg. Recovery was determined to be greater than 69% for all three analytes at 0.5 ng/mg, 5 ng/mg, and 20 ng/mg of hair. This method is currently being used to quantitate LAAM, dinorLAAM, and norLAAM in rat hair obtained from dose response studies as well as in human hair from subjects involved in clinical treatment programs. (Supported by NIDA grants #DA07820 and #DA09096.)

#42 Cocaine and Metabolites in Human Greying Hair: Pigmentation Relationship by Ralston W. Reid*, Francine L. O'Connor, Alyce G. Deakin, Delores M. Ivery and John W. Crayton, Biological Psychiatry, Psychiatry Service, 116A7 Hines V.A. Hospital, Hines, IL 60141 U.S.A.

Objective. To assess differences in the binding of cocaine, cocaethylene, and benzoylecgonine among pigmented and senile white hairs of the greying human cocaine abuser. A sheath of greying hair located around the apex of the head was gathered between the thumb and index finger then cut and removed about 2 mm proximal to the scalp. The greying hair was divided into pigmented and senile white of equal weights and lengths then analyzed by gas chromatography/mass spectrometry chemical ionization (GC/MS-CI). Twenty-nine (29) such pairs were analyzed. Concentrations of cocaine, cocaethylene, and benzoylecgonine were determined for 29 paired hair samples.

Results. There were statistically significant relationships between pigmented vs. senile white hair of paired samples: cocaine ((N=29) 31.5 ± 30.2 vs. of 14.9 ± 19.8 ng/mg) $P < 0.0001$; cocaethylene (N=21) of 3.22 ± 5.0 ng/mg), 0.52 ± 0.88 mg/mg), $P < 0.0016$; and benzoylecgonine (N=29) of 5.1 ± 5.3 vs. for their statistical means and standard deviations 3.9 ± 4.8 ; $P < 0.005$, respectively.

Conclusion. We believe that the design of this study offers a unique opportunity to continue to examine the hair color/pigment issue in hair analysis forensic application studies. In comparing an individual's cocaine concentrations between his own pigment vs. senile white hair, we have attempted to

isolate the pigmentation-cocaine binding question. Here we assume equal systemic cocaine exposure to the follicle of each member of the same hair pair and we believe we have avoided further questions of validity such as external contamination, effects of hair treatments, racial differences, individual metabolism of cocaine and cocaine dosages. We believe these data support cocaine and cocaethylene binding to hair pigment.

#43 Determination Of Drug Exposure Using Hair: Application To Child Protective Cases by Douglas Lewis and Christine Moore*, U.S. Drug Testing Laboratories, Chicago, IL U.S.A.; Patrick Morrissey, Child Protective Services, Waterloo, IA U.S.A.

The use of hair as a specimen for the determination of drug use remains controversial. Scientists disagree upon whether environmental drug contamination (e.g. smoke) can be differentiated from actual drug use. Children whose parents use drugs (particularly crack) at home are considered to be at risk. Using the hair of the children to determine exposure gives extra credibility to the child protective services and allows them to remove children from dangerous households. Children are tested when there are credible reasons for suspecting drug exposure. In Blackhawk County, Iowa, this program was implemented in 1994, and since then many children have tested positively for drugs, the majority of the hair containing cocaine or methamphetamine. In some cases, cocaethylene and benzoylecgonine were also found in the hair of the children. While the presence of benzoylecgonine can be explained by exposure to crack smoke, the presence of cocaethylene suggests ingestion of cocaine and alcohol. Blackhawk County Juvenile Court have found the program to be so useful in helping children that they have extended hair testing from Child Protective allegation investigations to ongoing court cases and even delinquency hearings.

#44 Preliminary Findings of Hair Analysis in SIDS-Related Deaths by Jeri D. Roper*, Diana Garside, and Bruce A. Goldberger, University of Florida College of Medicine, Gainesville, FL 32608 U.S.A.; John E. Smialek, Office of the Chief Medical Examiner, Baltimore, MD 21201 U.S.A.; H. Ronald Zielke and Robert Vigorito, University of Maryland School of Medicine, Baltimore, MD 21201 U.S.A.

Maternal drug use during pregnancy has been linked to many deleterious effects in the neonate including sudden infant death syndrome (SIDS). Studies indicate intrauterine exposure to drugs alters central nervous system (CNS) maturation in the fetus, and may subsequently affect the regulation of respiration. Thus far, research in this area has been limited to epidemiological-based studies. To improve the detectability of drug exposure in SIDS cases, hair analysis was utilized.

Head hair samples were obtained from 16 infants; 11 SIDS cases and 5 controls (cause of death included asphyxia, myocarditis, and cardiomyopathy). The hair was decontaminated with methanol, pulverized, and subjected to methanol reflux. The hair extracts and washes were purified by solid-phase extraction, and assayed by gas chromatography/mass spectrometry. Specimens were analyzed for morphine, codeine, and cocaine analytes.

Hair analysis revealed the presence of cocaine and its metabolites including benzoylecgonine, ecgonine methyl ester, cocaethylene, and norcocaine in 5 (45%) of the SIDS samples and 1 (20%) of the controls. All samples were negative for morphine and codeine, and all washes were negative for opiate and cocaine analytes. Results of conventional post-mortem toxicological tests were negative for opiate and cocaine analytes.

The presence of cocaine and its metabolites in infant hair indicates exposure to cocaine through various routes including intrauterine exposure, ingestion of drug through breast-feeding or suckling, and/or environmental contamination.

This research was funded in part by a grant from the University of Florida Division of Sponsored Research and NIH contract number N01-HD-1-3138.

#45 Enantiomer Profile for Amphetamine Derived From the Precursor Compound Fenproporex by John T. Cody* and Sandra Valtier, Clinical Investigations, Wilford Hall Medical Center, Lackland AFB TX 78236-5319 U.S.A.

Fenproporex, an anorexic drug, is one of several compounds metabolized to amphetamine by the body and excreted in the urine. As a result, amphetamine positive samples may be the result of ingestion of that drug as well as illicit use of amphetamine. Definitive interpretation can be made by detection of the parent drug. Unfortunately, in the HHS program, testing for drugs other than those covered by the Guidelines is prohibited except under special circumstances, thereby preventing the testing of a sample for the presence of fenproporex. As a result, the possibility of the use of fenproporex giving rise to an amphetamine positive urine sample can not be assessed by the presence of the parent. Evaluation of the enantiomeric composition of the sample can provide powerful evidence concerning the possible involvement of fenproporex. The amphetamine derived from fenproporex is racemic and therefore both enantiomers are seen following its use.

After administration of a 10 mg dose of fenproporex to each of five human subjects, the enantiomeric distribution showed the d-enantiomer to be higher than the l-enantiomer for 14-32 hours. This is in striking contrast to what is typically seen following use of racemic amphetamine, where both enantiomers are initially nearly equal in concentration followed shortly thereafter by the l-enantiomer predominating. Predominance of the d-enantiomer corresponded with the presence of the parent compound. As the parent drug disappeared, the amphetamine enantiomer composition changed, crossing over to have the l-enantiomer predominate compared to the d-enantiomer. This finding of atypical enantiomer distribution will greatly assist in the evaluation of fenproporex involvement in amphetamine positive results.

#46 Radioimmunoassay and Kinetic Interaction of Microparticles in Solution Methods Compared for Drugs of Abuse Testing by Robert R. Roussel, Dartmouth College Medical School, Hanover, NH 03755 U.S.A.; Paula J. Underwood and Edwin K. Armitage*, Forensic Toxicology Drug Testing Laboratory, 2490 Wilson St., Fort George G. Meade, MD 20755 U.S.A.

For many years the Department of Defense (DoD) used radioimmunoassay (RIA) as its accepted method for the initial testing of urine specimens for drugs of abuse. Recently, DoD laboratories ceased using RIA and commenced using the Kinetic Interaction of Microparticles in Solution (KIMS). Our objective was to compare RIA and KIMS in their ability to detect specimens that would be confirmed positive by GC/MS. We performed our assays using methods and instrumentation approved by the DoD. KIMS testing was performed using an Olympus AU800. In this study, we compared RIA (Roche Abuscreen®) and KIMS (Roche Abuscreen® OnLine™) for cannabinoids and cocaine metabolite detection by screening approximately 20,000 specimens for cannabinoids and 10,000 specimens for cocaine. Urine specimens were received from soldiers in the U.S. Army. Urine specimens that tested positive by either methodology were confirmed by gas chromatography/ mass spectrometry (GC/MS). The two tests were comparable for cocaine metabolite detection. RIA detected 99.1% of the confirmed cannabinoid-positive specimens versus 88.1% for KIMS; however, RIA also yielded 8.1% unconfirmed positive specimens. The confirmed positive specimens detected by RIA, but not KIMS, had concentrations of the 11-nor-9-carboxy-D⁹-THC at or below 30 ng/mL. Our laboratory prefers the KIMS testing because of the economic gains and improvement in the efficiency of testing. The philosophy of the DoD program is not one of zero tolerance but one of deterrence. The deterrent effect of the program is not appreciably altered with the new testing.

#47 An Abuscreen® OnLine Immunoassay for the Detection of LSD in Urine on the Cobas Integra Clinical Analyzer by Krystyna Goc-Szkutnicka, Maria G. Bates, Sharmila Honasoge, Alan J. McNally* and Salvatore J. Salamone, Roche Diagnostic Systems, Inc., Somerville, NJ 08876 U.S.A.

We have developed an Abuscreen® OnLine Immunoassay for the detection of LSD in human urine on a new clinical analyzer, the Cobas Integra. The Cobas Integra has the capability of fully automating a variety of homogenous assays for the detection of electrolytes, abused drugs, chemistries, specific proteins, and therapeutic drugs. This homogenous immunoassay for LSD is based on the Kinetic Interaction of Microparticles in a Solution (KIMS). Drug content in a urine sample is directly proportional to the inhibition of microparticle aggregation. The assay cutoff is 0.5 ng/mL, and the clinical sensitivity for the detection of LSD and its metabolites in human urine is equivalent to the LSD Abuscreen RIA. Test results are reported in a qualitative format, with absorbance values equal to or less than the cutoff, considered positive.

The performance of the OnLine LSD reagents on the Cobas Integra was evaluated using the following criteria: precision, clinical performance, correlation to GC/MS/MS, assay specificity (848 presumptive negative samples), cross-reactivity, and limit of detection (LOD). The intra (n=20) and inter (n=100) assay precision had %CVs values of ≤4.25 % at 0.25 ng/mL, 0.5 ng/mL, and 0.75 ng/mL. Twenty eight GC/MS/MS positive samples were analyzed on the Cobas Integra. All samples were positive. Eight hundred and forty-eight presumptive negatives were also analyzed on the Integra; 845 were negative and 3 were positive (0.35% false positive rate). Cross-reactivity studies showed a greater than 35% cross-reactivity to nor-LSD and 2.5% to iso-LSD. The LOD was found to be 0.064 ng/mL.

In summary, the Abuscreen LSD OnLine homogeneous, non-isotopic immunoassay on the Cobas Integra meets the sensitivity requirements for the detection of LSD and its metabolites in human urine.

#48 Homogeneous Cocaine Metabolite Enzyme Immunoassay with 150 and 300 ng/mL Cutoff by Parisa Khosropour*, Sean Lanyi and Andrew Lin, Diagnostic Reagents, Inc., Sunnyvale, CA 94086 U.S.A.

The National Institute on Drug Abuse (NIDA) requires a 300 ng/mL cutoff concentration for the cocaine metabolite immunoassay for the urine drug screening program. The Department of Defense (DoD) has adopted a 150 ng/mL as the cutoff value. We have developed a sensitive homogeneous EIA for the detection of benzoylecgonine in urine at both 150 and 300 ng/mL cutoff concentrations. The assay is based on the competition of the drug in urine and a drug-labeled enzyme conjugate for a fixed amount of anti-benzoylecgonine antibody. Enzyme activity measured at 340 nm is proportional to the drug concentration in urine. The ready-to-use liquid reagents require no preparation. The assay is readily adaptable to high volume analyzers. The performance is summarized below:

Sensitivity: 30 ng/mL

Calib./Control	Precision: Within-run (n=20)		Run-to-run (n=12)	
	Mean±S.D.	CV	Mean±S.D.	CV
Negative	330±1.89	0.6%	334±3.48	1.0%
100 ng/mL	389±2.12	0.5%	384±4.22	1.1%
150 ng/mL	418±1.51	0.4%	420±3.02	0.7%
225 ng/mL	451±2.44	0.5%	448±2.76	0.6%
300 ng/mL	483±2.70	0.6%	484±3.53	0.7%
375 ng/mL	499±3.54	0.7%	496±4.07	0.8%

A 100% agreement was found with a commercial cocaine EIA assay using 120 clinical specimens. The DRI assay is rapid and convenient for the detection of cocaine metabolites in urine.

#49 Serum Tox Assays: Benzodiazepines and Barbiturates and Tricyclic Antidepressants by Lan-Thanh Huynh*, Tom Chia, Andrew Lin and Parisa Khosropour, Diagnostic Reagents Inc., Sunnyvale, CA 94086 U.S.A.

DRI has developed homogeneous enzyme immunoassays for the detection of benzodiazepines, barbiturates, and tricyclic antidepressants in serum (Serum Tox). All assays are optimized to detect most of the compounds in the benzodiazepine, barbiturate, and tricyclic antidepressant drug classes. The assays are based on the competition of a drug-labeled enzyme and the free drug in the sample for a fixed amount of antibody. The liquid ready-to-use reagents require no preparation and are applicable to various clinical chemistry analyzers. Drug concentrations (ng/mL) in the calibrators and the assay performance are summarized below:

	Secobarbital	Diazepam	Nortriptyline
Negative	0	0	0
Cal 1	500	25	150
Cal 2	1000	50	300
Cal 3	3000	100	500
Cal 4	6000	200	1000

Calibrator	Benzodiazepines		Barbiturates		Tricyclics	
	Within-run	Between-run	Within-run	Between-run	Within-run	Between-run
Negative	330±2.0(0.6%)	330±0.8(0.2%)	217±1.9(0.9%)	216±2.7(1.2%)	357±3.1(0.9%)	346±3.9(1.1%)
Cal 1	364±3.7(1.1%)	363±1.5(0.4%)	273±1.1(0.4%)	270±1.6(0.6%)	377±2.1(0.6%)	369±3.9(1.1%)
Cal 2	408±3.7(0.9%)	404±1.7(0.4%)	311±2.5(0.8%)	311±1.9(0.6%)	399±4.7(1.2%)	387±2.8(0.7%)
Cal 3	480±2.1(0.4%)	482±0.9(0.2%)	371±3.8(1.0%)	371±1.6(0.4%)	434±2.9(0.7%)	430±2.5(0.6%)
Cal 4	554±3.9(0.7%)	552±1.4(0.3%)	431±3.3(0.8%)	437±3.5(0.8%)	469±4.8(1.0%)	460±3.6(0.8%)

Precision: Mean±S.D.(%CV); within-run:n=20; between-run: n=12

DRI serum tox calibrators are designed for use with each respective assay to establish a calibration curve for semiquantitative determination of the analyte concentration. Calibrator 2 is intended to be used as a qualitative cutoff reference. In summary, DRI reagents are sensitive in detecting benzodiazepines, barbiturates, and tricyclic antidepressants in serum samples.

#50 High Sensitivity CEDIA® DAU Benzodiazepine Assay Utilizing b-Glucuronidase by Neal F. Bellet*, Heidi A. Scholz, Michael L. Opel, Heidi Rytter, F. Roark Galloway and Pyare Khanna, Boehringer Mannheim Corp., 2400 Bisso Lane, Concord, CA 94524 U.S.A.

The addition of b-glucuronidase to the CEDIA DAU Benzodiazepine assay results in a unique approach to enhancing the sensitivity of a homogeneous enzyme immunoassay for the detection of benzodiazepines in urine. The b-glucuronidase is added directly to the R1 reagent, thereby obviating the need to individually treat samples with b-glucuronidase prior to performing the analysis for the presence of Benzodiazepines. In the assay, the CEDIA DAU benzodiazepine assay reagents are prepared as directed in the package insert, and then 5 µL of b-glucuronidase (Boehringer Mannheim Biochemicals) are added per mL of R1 reagent. The assay is performed on the Hitachi 717 as follows: 3 mL sample plus 130 mL Reagent 1 containing EA, antibody, and b-glucuronidase are incubated for 5 min, then 130 mL Reagent 2 containing ED-drug conjugate and substrate (CPRG) is added. The subsequent changes in absorbance are used to determine the concentration of drug in the sample. During the first 5 minute incubation the glucuronide conjugated benzodiazepine metabolites are cleaved to yield products that are much more immunoreactive than the conjugated benzodiazepines.

The addition of b-glucuronidase has no impact on the assay range, separation from negative to the 200 ng/mL cut-off, precision throughout the assay range, interference from endogenous compounds, and stability of the reagents. Cross-reactivity to oxazepam glucuronide increases from 0.2% to 26%, cross-reactivity to temazepam glucuronide increases from 1.5% to 27%, and cross-reactivity to lorazepam glucuronide increases from 1.1% to 47%. Sensitivity to GC/MS confirmed benzodiazepine positive urine samples containing oxazepam, temazepam, lorazepam, flunitrazepam, or clonazepam are increased 20% to 1500% depending on the drug and the degree of conjugation of the parent drug in the sample.

The CEDIA DAU benzodiazepine assay provides an automated and effective method to screen for the presence of benzodiazepines in urine. The addition of b-glucuronidase to the reagents results in a very sensitive high volume method to screen for the presence of Benzodiazepines in urine.

#51 Comparison of the Behring Syva Emit[®] LSD Assay and DPC LSD RIA on 483 Patient Urines by Alain G. Verstraete*, Tanja R. Troch. Laboratory of Clinical Biology - Toxicology, University Hospital, De Pintelaan 185, B-9000 Gent, Belgium.

We analyzed 483 patient urine samples with the Behring Syva Emit[®] LSD assay (adapted to a Hitachi 917 analyzer) and the Diagnostic Product Corporation Coat-a-Count[®] LSD assay. The cut-off concentration for both assays was 500 pg/mL LSD. Confirmation was done by GC-MS on a HP 5980 GC with a 5970 MSD (SIM mode) after extraction with Bond Elut Certify columns and derivatization with BSTFA. The detection limit was 250 pg/mL LSD.

Twenty samples were positive by both RIA and EMIT and 31 samples were positive by EMIT alone. Due to the limited volume of sample available, only 25 samples could be analyzed by GC-MS. Most samples that were positive by EMIT and RIA were confirmed by GC-MS. Out of 18 samples that were positive by EMIT, but not by RIA (E+R-), none could be confirmed by GC-MS. Although some of these samples could contain LSD concentrations lower than the detection limit of our GC-MS method, we suspect interference by other drugs. Drugs that caused a positive EMIT LSD

result in spiked urine specimens and were also found in one or more of the E+R- samples were fluoxetine, haloperidol, metoclopramide, paroxetine, pipamperone, promethazine, sertraline, and tiapride. These drugs are likely candidates for causing false positives. Preliminary quantitative results show that 0.4 µg/mL metoclopramide yielded a positive EMIT LSD result; 2.3 and 5.8 µg/mL metoclopramide were found in E+R- urine specimens. Pipamperone caused a positive at 6 µg/mL, while 12.4 and 13.8 µg/mL were found in E+R- urine specimens.

Further work must be done to explain the large number of E+R- results that we observed.

#52 FPIA Opiate Analysis in Vitreous Humor as a Tool for Screening Post Mortem Cases by Flor R. Mattos*, Institute of Forensic Sciences of Puerto Rico, P O-Box 11878, San Juan, Puerto Rico; 00922-1878.

The purpose of this paper was to ascertain the suitability of vitreous humor (VH) as a screening sample for opiates in autopsy cases. The correlation between opiate concentration in VH obtained by FPIA and morphine concentration found in blood by GC/MS was evaluated. Based on our findings, a cut-off value was established that will serve as a guideline for further GC/MS analysis in blood.

This study was performed in two stages. During the first stage, data obtained from autopsy cases of drug abuse related deaths was evaluated. In all cases, VH was analyzed for opiates by FPIA, and blood was analyzed for total morphine by GC/MS using solid-phase extraction after hydrolysis with β -glucuronidase.

Correlation between FPIA analysis in VH and total blood morphine concentration was very poor. Nonetheless, we found that morphine was found in 93% of the cases with FPIA concentration above 100 ng/mL, in 91% of the cases with FPIA concentration between 80-99 ng/mL, and in 80% of the cases with FPIA between 50 to 79 ng/mL.

The cut-off was set at 80 ng/mL and careful evaluation was highly recommended with those cases with an FPIA result ≥ 50 ng/mL before a decision was made to proceed with GC/MS analysis of blood.

The second stage of this study was initiated to determine if the same trend was observed when analyzing for free morphine. This time both VH and blood were analyzed by GC/MS when the FPIA obtained was ≥ 50 ng/mL. Again the correlation was poor but in 87% of the samples free morphine was found. With few exceptions, VH morphine concentrations were generally lower in VH than blood.

Our conclusion from the evaluation of this data is that VH opiate analysis by FPIA may be useful as a screening tool for opiate analysis. Nevertheless, careful evaluation of the circumstances and other specimens available is recommended before further analysis were mandated.

#53 Adaptation of the Diagnostic Reagents Incorporated (DRI) Cotinine Enzyme Immunoassay (CEI) for Urinalysis Using the Bayer Chem 1⁺ Analyzer by Joseph E. Manno^{1,2,3}, Michelle C. Dempsey¹, Barbara R. Manno^{1,3}, Imad K. Abukhalaf^{1,2}, Terry C. Davis^{2,4}, Azeem A. Syed⁵. Center of Excellence for Clinical and Forensic Toxicology¹, Departments of Medicine², Psychiatry³ and Pediatrics⁴, Louisiana State University Medical Center, Shreveport, LA, 71130-3932 U.S.A. and Diagnostic Reagents, Inc⁵, Sunnyvale, CA U.S.A.

For this procedure, the reagents for the CEI were used as supplied by the manufacturer. The Chem 1⁺ analyzer was calibrated using 25 (100 diluted with 0), 100, 250, 500, 1000 and 2000 ng/mL calibrators supplied by DRI. The Chem 1⁺ calculated the best fit as a log-logit cubic with a zero intercept of -3 and r^2 of 0.995. (Note: The Chem 1⁺ does not use the 0 calibrator for calculation purposes). The Chem 1⁺ was set to report values only on specimens calculated to contain at least 25 ng/mL of cotinine. Forty specimens collected from clients enrolled in a smoking cessation study at our institution were analyzed using the above procedure and by DRI using their recommended procedure on a Hitachi 717 Analyzer. Twenty-four of the specimens assayed on the Chem 1⁺ tested at less than 25 ng/mL. All but 5 of these specimens when assayed by DRI tested at less than 25 ng/mL (exceptions: 38.6, 68.6, 57.2, 47, 49.7 ng/mL). Concentrations for the 40 specimens analyzed ranged from 28.8 ng/mL to 968 ng/mL and there was no difference between the assay concentrations by paired t-statistics. The coefficient of variation at 250 ng/mL was 2.1%. Using this procedure, over 6000 tests per kit can be realized. Our data indicates that the DRI Cotinine Enzyme Immunoassay can be used as constituted by the manufacturer on the Chem 1⁺ Analyzer. For maximum accuracy on the Chem 1⁺ analyzer, the concentration cutoff should be greater than 100 ng/mL.

#54 Evaluation of a Digoxin II Assay by a MEIA Technique on the AxSYM System by Gerard Meenan*, Michael Lehrer, Helen McNally and Christine Fanelli, Department of Pathology, Long Island Jewish Medical Center, New Hyde Park, NY 11042 U.S.A., Marie Canavan, Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL 60064 U.S.A.

A Microparticle Enzyme Immunoassay (MEIA) method for digoxin utilizing Abbott's AxSYM System, a large, automated random/continuous access immunoassay analyzer, was evaluated. The assay is based on an antibody-antigen complex bound to microparticles in a matrix cell. The AxSYM pipets an 81 µL serum sample and anti-digoxin coated microparticles into a reaction vessel. An aliquot of the drug-polyclonal antibody complex is transferred to a matrix cell. The instrument then delivers digoxin:alkaline phosphatase conjugate to the matrix cell. Conjugate binds to remaining sites on the antibody coated microparticles. A fluorogenic substrate (4-methylumbelliferyl phosphate) is added, thus removing any unbound conjugate and providing substrate for bound conjugate to cleave the phosphate bond to yield 4-methylumbelliferone. The fluorescent product is measured by the AxSYM's MEIA optical assembly.

Comparison of 64 AxSYM Digoxin II patient results to Behring Diagnostics OPUS results yielded good agreement and reproducibility (correlation coefficient = 0.9669). A patient receiving Digibind therapy demonstrated elevated Abbott TDX digoxin levels, whereas, the AxSYM and OPUS digoxin assays yielded similar and lower results. The random access feature of the AxSYM and its ability to process samples without pretreatment was found to be beneficial.

#55 Validity Testing of the Triage[®] and Testcup[®] Immunoassay Systems for Drugs of Abuse by *Amanda J. Jenkins*, Rosalind Jones, and Edward J. Cone, Addiction Research Center, DIR, NIDA, NIH, Baltimore, MD U.S.A.*

A validity assessment study was performed on the Biosite Diagnostics Triage[®] Intervention Panel for drugs of abuse and the Roche Diagnostic Systems Ontrak Testcup[®] urinalysis panel. The Triage is a qualitative competitive binding immunoassay procedure utilizing ASCEND[™] Multimunoassay (AMIA[™]) technology for the detection of five drug classes in human urine. Triage cutoff concentrations for amphetamines, cocaine, opiates, PCP, and THC, were 1000, 300, 300, 25, and 50 ng/mL, respectively. A positive specimen produced a magenta colored bar adjacent to the drug name on the test device, whereas negative specimens resulted in no color. Testcup qualitative assays are based on the principle of microparticle capture inhibition for the detection of cocaine, THC, and morphine in human urine, with cutoff concentrations of 300, 50, and 300 ng/mL, respectively. In this assay, if the specimen was negative a blue band was formed in the reading panel. A positive specimen caused the reading membrane to remain white.

These assays were evaluated utilizing a urine specimen set consisting of: clinical urine samples (N=231); drug free urine samples fortified with standards (N=56); drug free urine samples (N=21); and drug free urine samples fortified with potential interfering substances (N=48). The specimen set was assayed in random order under blind conditions by the Testcup, Triage, and by GC/MS. Testcup and Triage results were determined independently by two readers. Results were interpreted as positive or negative for each drug class for both tests. All drug free urine samples were interpreted as negative by the 2 readers for all drugs detectable by the two on-site tests. Ephedrine/pseudoephedrine/phenylpropanolamine (1 mg/mL) and dextromethorphan resulted in false positive results for the Triage assay for amphetamine and PCP. The readers agreed on an average of 99% of the results. Overall, both tests were easy to perform, with results available within 10 to 15 min after sample application. Results remained stable for the Testcup for a minimum of 72 h whereas the reading panel of the Triage turned a deep magenta color within 30 minutes, preventing further reading.

Acknowledgements: The authors acknowledge supply of kits for this study by Biosite Diagnostics, Inc. (San Diego, CA) and Roche Diagnostic Systems (Somerville, NJ).

#56 CEDIA: A Semi-Quantitative Screening Method by *Patricia A. Pizzo* and Stacey L. Nash, Laboratory Specialists, Inc., Belle Chasse, LA U.S.A.*

CEDIA is the registered trade mark of an homogeneous enzyme immunoassay marketed by Boehringer Mannheim Corporation. The objective of this study was to determine the feasibility of using CEDIA as a semi-quantitative assay. Standard validation protocol was used. The assays evaluated for this study were benzoylcegonine (BE), marijuana (THC), and phencyclidine (PCP).

Linearity was determined by using GC/MS calibrators manufactured by Biopool. The assays were found to be linear between the following levels: THC, 6 - 100 ng/mL; BE, 79 - 915 ng/mL; PCP, 12 - 150 ng/mL.

Accuracy and precision at cutoff (THC 50 ng/mL, BE 300 ng/mL, and PCP 25 ng/mL) were measured using a Roche ONLINE cutoff calibrator. The results are as follows: Precision as % CV, THC 2.66, BE 4.12 and PCP 1.85; Accuracy as 1 SD, THC 1.16, BE 13.9 and PCP 0.5. Cross reactivity and interference studies were also performed. Comparison of CEDIA semi-quantitation to GC/MS quantitative results were performed on over 100 samples for each drug. The CEDIA BE and PCP results were within $\pm 20\%$ of the GC/MS assayed values for at least 75% of the samples assayed. The THC GC/MS value ranged from 29-61% of the CEDIA value with a mean value of 44% of the CEDIA semi-quantitative value.

The data provided sufficient documentation to use the CEDIA screening technique in semi-quantitative mode as a range finder for GC/MS. CEDIA screening values can be used to arrange samples in increasing concentration order as well as allowing the appropriate dilutions to be performed prior to GC/MS analysis. This procedure saves GC/MS analysis time and assists in preventing carryover.

#57 Use of Stable Methemoglobin Preparations for Validation of Carboxyhemoglobin Measurement in Postmortem Specimens by *Thomas D. Shupe, Mark D. Scott, Rong-Jen Hwang, Thomas A. Swift and Thomas G. Rosano*, Department of Pathology and Laboratory Medicine, Albany Medical Center, Albany, NY 12208 U.S.A.*

Carboxyhemoglobin (HbCO) measurement in the medical examiner setting is widely performed by spectrophotometric methods and to a more limited extent by gas chromatography. While it is known that spectrophotometric HbCO methods vary in their interference from methemoglobin (metHb) and that pre-analytical reduction of metHb with dithionite may be required, validation and quality control of HbCO measurement is limited by lack of stable metHb control materials. We have developed a technique to prepare stable pools of metHb and HbCO in the range of concentrations encountered in postmortem specimens and to challenge postmortem HbCO measurement techniques employing dithionite reduction and CO-oximetry.

MetHb was generated by oxidation of hemoglobin (Hb) with 6.0 mM NaNO₂ in whole blood. Cells were then washed with 0.9% NaCl, lysed by freeze/thaw, and Hb isolated by sephadex G-25 exclusion chromatography with antibiotic preservation (100 IU penicillin and 0.1 mg streptomycin/mL). HbCO was prepared from unoxidized Hb by tonometry with carbon monoxide. Refrigerated preparations showed stable levels of metHb (73.2% \pm 1.3SD) and HbCO (91.6% \pm 2.7SD) over a 3 week period of time, allowing quality control materials with a broad range of metHb and HbCO levels to be prepared.

Analysis of metHb controls by the Ciba Corning 270 CO-oximeter showed a positive metHb interference in the HbCO measurement as high as 26% \pm Hb. The interference was unaffected by the actual HbCO content of the sample but showed a dose relationship to the metHb level. Parallel studies with the Instrumentation Laboratories 282 CO-oximeter showed a negative interference from metHb. Evaluation of 14.4 mM dithionite pretreatment showed that metHb reduction was time dependent and remained incomplete after 2 hrs. Residual metHb resulted in an apparent increase in HbCO by the model 270 as high as 5% \pm Hb but a negligible effect on the 282 CO-oximeter. We conclude that metHb interference in HbCO determinations varies with instrumentation, the concentration of metHb in postmortem blood, and the efficiency of dithionite pretreatment. These variables indicate the need for stable metHb control materials for the validation of HbCO measurement in postmortem blood.

#58 Combined Toluene, Acetone and Methyl Ethyl Ketone Accidental Intoxication Resulting in One Fatal and One Nonfatal Outcome: Presentation of the Case and Review of the Literature by *Aristidis Tsatsakis**, *Manolis Tzatzarakis*, *Manolis Michalodimitrakis*, *Nicolaos Trikiliis*, *Department of Forensic Pathology, and Unit of Toxicology, Medical School, University of Crete and University General Hospital of Heraklion, Heraklion, Voulas 71409, Greece*, *George Troulakis*, *Dolapsakis*, *Intensive Care Unit and Department of Radiology, Venizelion, General Hospital, Knossos, Iraklion, Crete, Greece.*

Fatalities due to intentional or accidental acute exposure to toluene fumes have been previously reported in the literature. The present case reports the accidental acute exposure of two male painters (18 and 30 y.o.) to fumes of paint diluent mixture containing toluene, acetone and methyl ethyl ketone (60:15:15, w/w) during their work in an underground reservoir. Both workers were found unresponsive by colleagues and immediately transferred to a local hospital. On admission, the younger one was pronounced dead, while the older remained in the intensive care unit for 3 days followed by four days in the internal medicine ward. Toluene (TL), acetone (ACT), and methyl ethyl ketone (MEK) concentrations of blood samples taken from the survivor on admission were 6, 30, and 40 mg/mL. Postmortem toxicological analysis of the dead worker revealed blood levels of TL, ACT, and MEK of 12, 90, and 80 mcg/mL respectively. The solvent levels in the liver, kidney, lung, brain, testis, and gland were quantitated and showed similar distribution of the chemicals with highest levels found in brain and liver. Quantitation and confirmation was performed by gas chromatography using different capillary and packed columns (e.g. 10' x 1/8' stainless steel, Supelco Carbopack B/30% SP-1500, or C1500, 10% CW HP 80/100).

General procedure: Approximately 2 g of specimen (tissue or fluid) was mixed with 100 mL of IS solution (2-butanol) in an 8 mL screw-capped vial. The vials were thermostated at 60°C for 40 min and the gas vapor phase (0.25 mL) was injected into the GC.

The reported cases indicate the need of prophylactic measures that should be taken when using paint diluents indoors.

#59 Death from an Overdose of Phenylbutazone by *David M. Andrenyak**, *Matthew L. Cheever*, *John D. Laycock* and *Dennis J. Crouch*, *Center for Human Toxicology, Univ. of Utah, Salt Lake City, UT 84112 U.S.A.*; *Floyd Fantelli*, *Eastern Idaho Regional Medical Center, Idaho Falls, ID 83404 U.S.A.*

This report describes the toxicological findings in a case involving an overdose of phenylbutazone, an anti-inflammatory drug used in veterinary and human medicine. A 40 y.o. woman was found unconscious and having seizures. Her husband alleged that she had taken 30-40 pills from a bottle that was labeled Butatabs-E (phenylbutazone - 1 g/tablet). The woman was admitted to a hospital and died 24 hours later. At autopsy, the small intestine was found to contain 200-300 mL of a liquid containing white granular particles. Samples of the intestinal contents and vitreous fluid was submitted to the Center for Human Toxicology for analysis. The samples were screened for volatiles, drugs of abuse, acidic, basic, and neutral drugs. In the screening, phenylbutazone was identified in the intestinal contents by electron impact GC-MS and flow injection electrospray mass spectrometry. For quantitation of the phenylbutazone, the samples were extracted with chloroform under acid conditions with mephobarbital as the internal standard. The extracts were analyzed by electron impact GC/MS. Since only a sample of the entire small intestine contents were received and the sample was very viscous, serial dilutions of a 1 g aliquot of the small intestine contents were prepared and analyzed. Phenylbutazone was detected in the vitreous fluid at 239 ng/mL and in the small intestine contents at 83 mg/g. The density of the intestinal fluid was estimated to be 1.2 g/mL. Using this density and the volume of the small intestinal fluid, the total amount of phenylbutazone in the intestinal fluid was estimated to be between 20-30 g. This amount of phenylbutazone was equivalent to 20-30 pills. The cause of death was attributed to an overdose of phenylbutazone. The manner of death was ruled a suicide.

#60 Postmortem Tissue Distribution of Venlafaxine: Six Case Studies by *Robert D. Budd* and *Daniel T. Anderson**, *Los Angeles County - Department of Coroner, 1004 N. Mission Road, Los Angeles, CA 90033 U.S.A.*

Venlafaxine is a new antidepressant agent which entered the United States market in 1994. To date, there is little or no information in the literature regarding postmortem levels. During 1995, the Los Angeles County Coroner's Toxicology Laboratory encountered six venlafaxine cases, with both therapeutic and toxic levels. In all six cases, postmortem venlafaxine tissue levels were determined.

The analysis of venlafaxine from postmortem specimens (2 mL sample size) consisted of an n-butylchloride basic extraction procedure with screening and quantitation on a GC/NPD. Linearity was achieved from 0.10 mg/L to 3.0 mg/L with the limit of quantitation being 0.10 mg/L. Confirmation of venlafaxine was performed on a GC/MS by comparison with an analytical standard.

Four of the cases were drug overdoses and in the other two, venlafaxine was present, but not directly related to the cause of death. In the two overdoses where the drug was directly linked to the cause of death, the concentrations of venlafaxine was distributed in the tissues as follows: heart blood, 76 and 13 mg/L; liver, 443 and 102 mg/kg; bile 365 and 86 mg/L; gastric 21 and 385 mg total. To our knowledge, these are the first reported fatalities involving the anti-depressant venlafaxine.

#61 Two Overdose Fatalities Involving Health Care Professionals: Postmortem Tissue Distribution of Fentanyl by *Daniel T. Anderson**, *Los Angeles County - Department of Coroner, 1104 N. Mission Road, Los Angeles, CA 90033 U.S.A.*

Fentanyl is a potent, short-acting narcotic analgesic widely used as a surgical anesthetic and easily accessible to licensed health care professionals. The Los Angeles County Coroner's Toxicology Laboratory recently encountered two cases, one involving an anesthesiologist, and the other, an internist. Both committed suicide with a lethal injection of fentanyl.

The analysis of fentanyl from postmortem specimens (3 mL sample size) consisted of an n-butylchloride basic extraction procedure and quantitation performed on a gas chromatograph-mass spectrometer in the selected ion monitoring mode. The fentanyl ions monitored were 245, 146, and

189 m/z, and the internal standard, D₅-fentanyl, were 250, 151 and 194 m/z (quantitation ion in italics). The limit of detection/quantitation of the method was 1.66 mg/L, and the assay was linear from 1.66 mg/L to 500 mg/L.

The postmortem tissue distributions of fentanyl were determined in both cases. In the case that involved the anesthesiologist, the fentanyl concentrations were: heart blood, 8.7 mg/L; liver, 61.8 mg/kg; vitreous, 7.0 mg/L; urine, 9.3 mg/L; and gastric, 3.1 mg total. The second case involving an internist, the levels were as follows: heart blood, 12.2 mg/L; liver, 75.6 mg/L; bile, 32.7 mg/L; urine, 11.8 mg/L; gastric, 5.3 mg total; and left arm needle site, 82.6 mg/kg.

#62 A Randomized, Controlled Clinical Trial of Oral Activated Charcoal in the Self Poisoned Patient by Kevin Merigian*, Kari Blaho, Mike Cockrell, Stephen Winbery and Lynda Park. Department of Emergency Medicine and Clinical Toxicology, UT Memphis, TN 38163 U.S.A.

The use of oral activated charcoal (OAC) for the treatment of overdose has long been considered the mainstay in the management of patients with oral drug overdose. The benefits of this unproven therapy are not known. We conducted a large, prospective randomized study on 1451 self-poisoned patients. All patients were randomly assigned to a treatment with OAC or observation on an even-odd day rotation. Exclusion criteria included the ingestion of mushrooms, crack cocaine, ethylene glycol, iron preparations, formaldehyde, methanol, heavy metals, or acetaminophen (>140 mg/kg). No patient underwent gastric evacuation in the emergency department. Outcomes were measured by length of ED stay, disposition, length of hospital stay, and complications in the clinical course of the patient. Of the 1451 patients, 403 (27%) received OAC. Most patients were discharged home from the ED, 153 were admitted to the hospital floor, and 60 were admitted to the ICU. Presenting vital signs were not different among the groups. There were no significant outcome differences between the groups. Results indicate that gastric decontamination by OAC does not enhance the clinical course of the self poisoned patient and that OAC is not required for safe management of these patients. Furthermore, overdose patients can be safely and effectively managed without gastric lavage or forced emesis.

#63 Pharmacokinetics and Pharmacodynamics of Orally Administered Diacetylmorphine Hydrochloride by Evelyne D. Gyr*, Daniel F. Bourquin, Thomas E. Lehmann and Rudolf M. Brenneisen, Institute of Pharmacy, University of Bern, Baltzerstrasse 5, CH-3012 Bern, Switzerland; Irene Hug, Opiat-verschreibung JANUS, Psychiatric University Hospital, CH-4004 Basel, Switzerland.

Within the "Heroin Maintenance Program" of the Swiss Federal Office of Public Health, the oral application of high-dosed diacetylmorphine hydrochloride (DAM) is discussed as an alternative to the traditional parenteral administration of DAM. The present work shows pharmacokinetic and pharmacodynamic data of a controlled clinical pilot study with two subjects after oral and intravenous administration of DAM. First, subject A and B received an intravenous dose of 220 mg and 190 mg DAM, respectively. The next day the subjects were administered a first oral dose of 400 mg DAM as capsule, followed 6 h later by a second oral dose of 400 mg DAM. At defined intervals blood samples were collected and pharmacodynamic parameters monitored by standardized visual analog scales (VAS). Immediately after centrifugation, the plasma samples were stabilized with 2 % NaF. After automated solid-phase extraction, DAM, 6-monoacetylmorphine (MAM), morphine (M), morphine-6-O-glucuronide (M6G) and morphine-3-O-glucuronide (M3G) were quantified by HPLC-DAD. The full data evaluation (bioavailability, psychological and physiological effects, etc.) of the oral application form was performed by comparison with the intravenous data. No DAM and MAM could be detected after the oral administration. The peak plasma concentrations of M, M6G and M3G after two oral doses of 400 mg DAM were 672 (1st dose) / 836 (2nd dose), 1446 / 1504 and 6117 / 6823 ng/mL for subject A and 1232 / 1004, 1902 / 1564 and 6569 / 5267 ng/mL for subject B, respectively. There was a "flash effect" reported by VAS after the oral administration, although it was lower than after the intravenous administration.

#64 In Vitro and In Vivo Smoking Experiments with Diacetylmorphine Cigarettes by Anna B. Stalder*, Thomas E. Lehmann, Daniel F. Bourquin and Rudolf M. Brenneisen, Institute of Pharmacy, University of Bern, Baltzerstrasse 5, CH-3012 Bern, Switzerland; Robert B. Hämmig, KODA-1, Department of Social Psychiatry, University of Bern, CH-3012 Bern, Switzerland.

In 1994, the Swiss Federal Office of Public Health started a novel "Heroin Maintenance Program". The pulmonary administration of sweet woodruff herb cigarettes impregnated with diacetylmorphine base (DAM, heroin) has been evaluated as an alternative to the intravenous application of DAM hydrochloride. First, in vitro smoking studies were performed to determine the release of DAM and pyrolysis products from the tobacco-free cigarette matrix. The smoke was trapped on a solid-phase column, and the eluate analyzed by HPLC-DAD. After the continuous smoking of cigarettes containing 100 mg DAM, 6.5 ± 0.4 % DAM (mean ± SEM, n = 10), 10.1 ± 0.6 % 6-monoacetylmorphine (MAM) and 3.5 ± 0.2 % morphine (M) could be found in the smoke. More realistic smoking conditions (4 puffs of 5 sec/min) led to recoveries of only 2.2 ± 0.3 % DAM, 5.5 ± 0.5 % MAM and 3.2 ± 0.4 % M. A controlled clinical pilot experiment was performed with 2 subjects who smoked 5 cigarettes containing 100 mg DAM under standardized conditions (4 puffs of 5 sec/min) over a period of 105 min. At defined intervals blood samples were collected and pharmacodynamic parameters monitored by visual analog scale (VAS). After automated solid-phase extraction, the stabilized (2 % NaF) plasma samples were analyzed by HPLC-DAD. The full data evaluation (bioavailability, psychological and physiological effects, etc.) of the pulmonary application form was performed by comparison with intravenous data (50 mg DAM hydrochloride). The peak plasma concentrations of DAM, MAM, M, morphine-6-O-glucuronide and morphine-3-O-glucuronide were found to be 563, 311, 267, 432, and 1818 ng/mL for subject A and 464, 516, 420, 660, and 3458 ng/mL for subject B, respectively. Psychological and physiological effects reported by VAS showed a good correlation with the plasma levels.

#65 Urinary Excretion Profiles of Psilocin After Oral Administration of Psilocybin by Felix Hasler*, Daniel F. Bourquin and Rudolf M. Brenneisen, *Institute of Pharmacy, University of Bern, Baltzerstrasse 5, 3012 Bern, Switzerland; Truls Bär and Franz X. Vollenweider, Psychiatric University Hospital Zürich, Lenggstrasse 31, 8029 Zürich, Switzerland.*

In a clinical study eight subjects received oral doses of 212 ± 3 µg/kg body-weight of psilocybin (PY). To study the urinary excretion of psilocin (PI), the first metabolite of PY, urine samples were collected for 24 h and PI was quantified by high performance liquid chromatography with column-switching and electrochemical detection (HPLC-ECD). Sample work up included protection of the unstable phenolic analyte with ascorbic acid, freeze-drying and extraction with methanol. The precision of the method was found to be $4.5 \pm 1.8\%$ (mean \pm SEM; n = 12) and the recovery for PI was $102.7 \pm 3.2\%$. Peak levels of PI were measured in samples of the 2-4 h collection period showing concentrations of 402 ± 96 ng PI/mL urine. The limit of detection (10 ng PI/mL urine) was usually reached 24 h after drug administration. $3.34 \pm 0.31\%$ (mean \pm SEM; n = 8) of the applied dose was excreted as free PI within 24 h. Addition of β -glucuronidase to urine samples and incubation for 5 h at 40°C led to two-fold higher concentrations of PI although 20% of the amount of unconjugated PI was decomposed during incubation. We conclude that in man PI is partially excreted as glucuronide and that enzymatic hydrolysis of urine samples leads to an increase of sensitivity of the HPLC assay.

#66 Comparison of Ante-Mortem And Post-Mortem Drug Concentration In 6 Cases Of Fatal Drug Overdosage by Robin A Braithwaite* and Keith A Hale, *Regional Laboratory for Toxicology, City Hospital, Birmingham, U.K.*

Interpretation of post-mortem blood concentrations in suspected cases of poisoning presents many difficulties. Two important factors are the post-mortem redistribution of drug, and the choice of blood sampling site.

We have investigated six recent cases of fatal drug overdosage where blood (plasma) specimens had been taken in the hospital prior to death as part of patient management. Blood was also obtained at during autopsy for forensic examination. The cases involved the following drugs: propranolol (1 case), paracetamol (acetaminophen) (3 cases), dextropropoxyphene (2 cases), dothiepin (2 cases), and amitriptyline (1 case).

Post-mortem blood specimens were taken from different sites according to the pathologists' routine practice. In all cases specimens were obtained from more than one site. In every case and for every drug there was an increase in the post-mortem blood concentration compared with the ante-mortem plasma drug concentration. The average percentage increases in post-mortem drug concentrations were as follows: propranolol (823%), paracetamol (282%), dothiepin (993%), nordothiepin (2472%), dextropropoxyphene (946%), nordextropropoxyphene (1321%), amitriptyline (477%), nortriptyline (556%). Specimens taken from the heart showed the highest increase (average 2145%) whereas specimens from the femoral region showed the lowest increase (640%), specimens from the sub-clavian region showed intermediate increases (776%). Great caution is required in the interpretation of post-mortem blood concentrations, even for specimens taken from recommended sites such as the femoral region.

#67 Permeation of Morphine and Morphine-Glucuronides Through a Vascular Wall by Gisela Skopp*, Rainer Lutz, Beate Ganßmann and Rolf Aderjan, *Institute of Legal Medicine, Voßstr. 2, 69115 Heidelberg, Germany.*

When measuring drug concentrations after death it is important to consider the phenomena of postmortem redistribution. There are only a few investigations on time dependent changes on drug concentration. To study the postmortem permeation characteristics of morphine, morphine-3-glucuronide, and morphine-6-glucuronide, a Chien-Valia diffusion chamber with vein samples clamped between the connecting flange surfaces was used. Dependence on temperature, initial substance concentration, direction of solute flow, and molecular structure is reported.

There was a marked influence of storage temperature only for the first 40 hours. The lag time increased with decreasing initial substance concentration. The permeation profile depended on the orientation of the vascular wall. The transvascular solute flux slightly increased in the following order: morphine < morphine-6-glucuronide < morphine-3-glucuronide. Although the diffusion cell is restricted in application to large veins, it appears to be a useful tool to elucidate some of the effects of postmortem redistribution.

#68 Deaths Involving Flunitrazepam: An Australian Experience by Michael D. Robertson, *National Medical Services, 2300 Stratford Ave., Willow Grove PA, U.S.A. and Victorian Institute of Forensic Medicine, Monash University, Melbourne Australia.*

Flunitrazepam (Rohypnol®) a premedication for surgery and prescribed night time hypnotic, is widely used clinically throughout the world, but is not available for medicinal use in the USA. Its use is however becoming more prevalent in the USA, due to increased illicit and 'recreational' use. Its prevalence may however be extremely conservative with many US laboratories not appreciating the importance of the 7-amino metabolite, especially in post-mortem toxicological investigations.

Post-mortem specimens were analyzed in 30 deaths involving flunitrazepam in Victoria, Australia. The concentrations of flunitrazepam and its major metabolite, 7-amino flunitrazepam were determined in a variety of post-mortem specimens by isocratic HPLC-UV. In only bile and urine was the parent compound detected. The concentrations of 7-amino flunitrazepam in the post-mortem specimens were: blood (0.06 mg/L), plasma (0.06 mg/L), vitreous humor (0.02 mg/L), urine (0.38 mg/L), bile (0.88 mg/L), and liver (0.11 mg/L). Plasma and liver contained 7-amino flunitrazepam concentrations similar to blood. Vitreous humor contained low concentrations of 7-amino flunitrazepam. Vitreous humor may be useful in decomposed cases, whilst bile and urine contained much higher concentrations, and are hence useful screening specimens.

These data suggest that 7-amino flunitrazepam can be detected in a variety of post-mortem specimens. 7-Amino flunitrazepam also gave a better indication of flunitrazepam use prior to death than did the parent compound. Consequently, analyses that do not determine the presence of 7-amino flunitrazepam cannot exclude the involvement or contribution of flunitrazepam in death.

#69 Qualitative Analysis of a Towel and a Sock for Isopropyl Alcohol to Aid in a Homicide Investigation by *Laureen J. Marinetti-Sheff^{*}, Michigan State Police Crime Lab Toxicology Subunit, 714 S. Harrison, E. Lansing, MI 48823 U.S.A.; Michael J. Caplan, The University of Michigan Department of Pathology, 1301 Catherine, Box 0602, Ann Arbor, MI 48109 U.S.A.*

We report an unusual homicide case that at first appeared to be a death due to ligature strangulation. However, an autopsy revealed no complex array of ligatures connecting her neck and extremities. Upon examination of the scene and also at autopsy there was very little evidence of a struggle. It became apparent that the victim was subdued or incapacitated in some way prior to being tied up. The medical examiner screened the vitreous fluid for drugs and volatiles suspecting chloroform. The lab results showed 110 mg/dL isopropyl alcohol (IPA) and 3 mg/dL acetone. Questioning of the suspect indicated that IPA may have been used.

A towel and sock were collected at the scene and packaged in paper bags from serological analysis. As soon as it was discovered that a volatile could be involved, the towel and sock were re-packaged in an airtight plastic bag and placed in a freezer. Unfortunately by this time, the towel and sock had completely air-dried. Despite this fact, the toxicology laboratory was able to demonstrate the presence of IPA in both the towel and the sock with headspace gas chromatography utilizing a blood volatiles method. The cause of death was determined to be smothering with acute IPA intoxication.

#70 Jin Bu Huan Poisoning: A Toxicological Analysis of Tetrahydropalmatine by *Daniel T. Anderson^{*}, Los Angeles County - Department of Coroner, 1104 N. Mission Road, Los Angeles, CA 90033 U.S.A.*

Tetrahydropalmatine or Jin Bu Huan Anodyne is an alkaloid found in plants of the *Carydalis* species. The drug is considered to be a natural herb and is a traditional Chinese medication with sedative and tranquilizing effects. It has been around for more than 1000 years as an analgesic, but only available in the United States for the past 10 years.

Documentation of the drug's involvement in patients is limited only to toxicity, not lethality. This paper describes a case study report involving a female who committed suicide with a lethal ingestion of tetrahydropalmatine in blood.

The analysis of tetrahydropalmatine consisted of an *n*-butylchloride extraction procedure with screening and quantitation on a GC/NPD. Confirmation of the drug was performed on a GC/MS by comparison of a purified Jin Bu Huan Anodyne Tablet.

The tetrahydropalmatine concentration detected in postmortem blood of a 50 y.o. female was 7.2 mg/L. The gastric levels suggested 3.8 g or 141 pills were ingested. This is the first reported fatality involving the drug, tetrahydropalmatine.

#71 Determination of Tramadol and its Metabolites in Postmortem Blood by *Kabrena E. Goeringer^{*} and Barry K. Logan, Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, 2203 Airport Way So. Suite 360, Seattle, WA 98134 U.S.A.*

Tramadol (T) is a centrally acting, binary analgesic which is neither an opiate-derived nor a non-steroidal anti-inflammatory drug. It is used to control moderate pain in several chronic pain settings, including osteoarthritis and post-operative cases. Used in therapy as a racemic mixture, the (+)-enantiomer weakly binds to the mu-opioid receptor, and both enantiomers inhibit serotonin and norepinephrine reuptake. The major active metabolite, *o*-desmethyl-tramadol (ODT), shows higher affinity for the mu-opioid receptor and has twice the analgesic potency of T. The synergism of these effects contributes to T's analgesic properties, with the (+)-enantiomer exhibiting a 10-fold higher analgesic activity than the (-)-enantiomer.

Analytical methods using gas chromatography/mass spectrometry and high performance liquid chromatography with photodiode array detection were utilized for determination of T and its metabolites. An *n*-butyl chloride extraction was followed by GC/MS analysis using a 5% phenylmethylsilicone column (30 m x 0.32-micron i.d.). The chloroform was then evaporated and the extracts reconstituted in methanol for HPLC-PDA analysis on a Merck RP Select B column. Analysis of nine blood samples from T-related deaths revealed concentrations ranging from 0.03-1.43 mg/L for T, 0.02-1.84 mg/L for *n*-desmethyl-tramadol (NDT), and 0.01-0.73 mg/L for ODT. In five cases, other drugs known to affect the central nervous system, including propoxyphene, dextromethorphan, amitriptyline, and diazepam were present. The possible role of serotonin syndrome in these deaths will be discussed.

#72 Salivary Excretion Kinetics and Saliva/Plasma Ratios of Methadone and Metabolite (EDDP) in Humans by *Pierre Marquet^{*1}, Patrick Mura², Hayat Lotfi¹⁻³, Yves Papet², Louis Merle¹ and Gérard Lachâtre¹⁻³, ¹Department of Pharmacology and Toxicology, University Hospital, F 87042 Limoges Cedex, France, ²Department of Toxicology, University Hospital, F 86000 Poitiers Cedex, France, ³Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, F 87025 Limoges Cedex, France.*

Contrary to most other drugs of abuse, methadone excretion in saliva has only been reported in two papers with different results. Lynn et al. (J. Chromatogr., 1977) found a S/P ratio between 3 and 10 after an intramuscular administration, while Kang and Abbott (J. Chromatogr., 1982) reported a mean value of 0.51 ± 0.13 , after oral administration. Salivary determination of methadone could be useful in methadone maintenance monitoring, either to verify the compliance with the treatment or for dose adaptation or dose fractionation. The aims of this study were: i) to determine the excretion kinetics of methadone and EDDP in saliva, ii) to evaluate the saliva/plasma ratio and the possibility of using saliva instead of plasma for methadone treatment monitoring and adaptation.

Three male methadone maintenance patients, hospitalized for a medical intercurrent cause, volunteered to undergo regular blood and saliva sampling over 24 hours after an oral administration of methadone. Methadone and EDDP were determined by a GC-MS assay with deuterated internal standards.

Methadone concentrations in saliva were similar to plasma concentrations after elimination of initial oral contamination. The saliva concentrations peaked between 5-8 h. At 24 h, concentrations ranged from 267 ng/mL to 1208 ng/mL. The S/P ratio varied with time from 1.74 to 10.53, with a mean value of 5.86 ± 2.74 . EDDP appeared in saliva at 1 h, reached a maximum after approximately 4 to 12 h, and presented a mean S/P ratio of 1.00 ± 0.60 .

Thus, salivary methadone concentrations may be used for maintenance monitoring and possibly for dose adaptation.

#73 Predicting New Marijuana Use from Creatinine Normalized 11-Nor-9-carboxy-D9-tetrahydrocannabinol (THCCOOH) Concentrations: Criteria Development and Validation by Marilyn A. Huestis* and Edward J. Cone, Chemistry and Drug Metabolism Section, ARC, NIDA, NIH, PO Box 5180, Baltimore, MD 21224 U.S.A.

Drug treatment specialists are frequently faced with the task of determining whether a second positive urine cannabinoid test reflects new marijuana use or residual drug excretion. Prediction of new use is difficult due to intrasubject variation in the concentration of urine over time; however, this variation can be minimized by normalizing the urine cannabinoid concentration (ng/mL) to the creatinine concentration (mg/mL). A positive urine test result is normalized by dividing the THCCOOH concentration by the creatinine concentration. A ratio is then calculated by dividing the normalized value of the later result by the earlier normalized result. In 1984, Manno, et al., proposed that a ratio ≥ 1.5 was evidence of new marijuana use. In the present study, we performed a Receiver Operating Characteristics (ROC) analysis on 1837 pairs of positive cannabinoid urine tests to determine optimal criteria for identifying new use. We systematically evaluated 26 different ratio criteria ranging from 0.1 to 2. Urine specimens were collected from six male subjects who smoked a single marijuana cigarette each week containing 0, 1.75%, or 3.55% THC in a controlled clinical setting. THCCOOH concentrations were measured by GC/MS and creatinine concentrations were measured by a modified Jaffe method. The number of new uses that were correctly identified (true positive, TP) or missed (false negative, FN) and the number of positive cases representing carryover that were correctly identified (true negative, TN) or misidentified as new marijuana use (false positive, FP) were determined for each ratio. Criteria were applied to specimens collected a minimum of 24 h apart. The best accuracy for prediction of new marijuana use was achieved with a normalized ratio ≥ 0.5 . The overall prediction accuracy was 85.4%, with 5.6% FP and 8.6% FN. The currently used criteria ≥ 1.5 was only 74.2% accurate, with 0.1% FP and 27.0% FN predictions. Lower FP percentages can be achieved by selecting higher ratios; however, reduced FP percentages were always attained at the expense of increased FN percentages. This validation study indicates that new marijuana use can be accurately predicted from normalized cannabinoid data by selection of criteria to minimize FP and FN results.

#74 Clinical Manifestations of Acute Cocaine Intoxication and Corresponding Levels from Patients Evaluated in an Inner City Emergency Department by Kevin Merigian*, Kari Blaho and Stephen Winbery, Department of Emergency Medicine and Clinical Toxicology, UT Memphis, Memphis, TN U.S.A.; Stephen A. Geraci, Department of Medicine, UT Memphis, Memphis, TN U.S.A.; Barry Logan, State Toxicology Lab, University of Washington, Seattle, WA U.S.A.

Obtaining blood cocaine concentrations from patients in an acute care setting is unusual. We report cocaine and metabolite levels along with corresponding clinical findings in 16 patients presenting to an inner city emergency department (ED) with the diagnosis of acute cocaine intoxication. Of these, 10 were male and 6 were female. The mean age was 26.7 ± 7.8 y, with a range of 16-44 y. Three patients smoked crack cocaine, 4 ingested crack to avoid arrest, 2 patients administered cocaine intravenously, and 2 insufflated the drug. One patient smoked crack and then ingested the remainder to avoid arrest. A severity of intoxication scale was developed that included cardiovascular parameters, CNS findings and results of physical exam. Of 16 patients, 8 were severely intoxicated, 7 exhibited moderate intoxication, and 1 was mildly intoxicated. Blood was collected for analysis for cocaine and metabolite levels as soon as possible after arrival to the ED, usually within 2 hours. After collection into tubes containing sodium fluoride, blood was immediately frozen and stored frozen and thawed immediately prior to analysis. Cocaine and metabolite concentrations were determined by an extractive alkylation/mass spectrometry procedure. In some instances, time-series samples were collected on patients. Of the 16 patients, only 4 patients were admitted to the hospital and none died. Several patients required large doses of benzodiazepines for agitation. Cocaine levels in blood ranged from 0 to 1.74 mg/L, levels of benzoylecgonine ranged from 0.32-4.57 mg/L, and ecgonine methyl ester levels ranged from 0-1.31 mg/L. Results from this preliminary study indicate that cocaine and metabolite concentrations vary widely depending on patterns of use and route of administration, and may not necessarily accurately predict the severity of the clinical findings.

#75 Predicting the Amount of Illicit Cocaine Use from Benzoylecgonine and Creatinine Concentrations in a Single Urine Specimen by Edward J. Cone*, K.L. Preston and J. Oylar, Addiction Research Center, DIR, NIDA, NIH, Baltimore, MD U.S.A.

Although there is no general relationship between urine drug concentration in individual specimens and dosage, there is a relationship between dose and maximum concentration. We evaluated the relationship between peak excretion of the cocaine metabolite, benzoylecgonine (BZE), in single specimens and dose in five human subjects following intravenous (IV) administration of known doses of cocaine hydrochloride (1-25 mg). Specimens were analyzed by GC/MS, and the ratio of urine BZE concentration to urine creatinine concentration was used to determine peak BZE excretion. Peak BZE excretion was significantly related to dose by simple linear regression according to the equation $y = mx + b$, where y = cocaine HCl dose (mg), x =

peak BZE excretion (ng/mg); m = slope and b = y intercept. The equation describing the relationship was as follows: $y = 0.002494x + 2.7788$ with $r = 0.907$. Prediction intervals (95% confidence limits) were described by the relationship:

$$Y \pm t_{0.95} s_{r,x} \sqrt{1 + \frac{1}{n} + \frac{(X_0 - \bar{x})^2}{\sum x^2}} = Y \pm 2.052 * 4.42 \sqrt{1 + \frac{1}{29} + \frac{(\text{observed } X - 3552.8)^2}{391060119}}$$

The equations were then applied to BZE concentrations of urine specimens collected from female and male illicit cocaine users ($N=17/\text{group}$) who were also in treatment for heroin addiction. The estimated minimum amount of cocaine HCl used by these subjects ranged from 55-6282 mg (mean = 1,393, median = 419) for females and 100-2,393 mg (mean = 479, median = 204) for males. The highest minimum estimated amount of cocaine consumed, 6282 mg, had an associated prediction interval of $\pm 1,153$ mg. Assumptions inherent in this method include: 1) a linear dose-concentration relationship; 2) consumption of cocaine in divided amounts over several hours-days. This method provides an estimate of the minimum amount of cocaine necessary to produce observed BZE/creatinine ratios in urine specimens of unknown history. Although this method needs further validation, it is designed to provide conservative estimates of the amount of recent cocaine consumption by illicit drug users.

#76 A Presumed Cocaine Induced Seizure: Case Report and Drug Concentrations by Stephen Winbery*, Kari Blaho and Kevin Merigian, Department of Emergency Medicine and Clinical Toxicology, UT U.S.A.; Barry Logan, State Toxicology Laboratory, University of Washington, Seattle, WA U.S.A.

Seizures induced by cocaine are relatively rare. So much so, that when they are associated with cocaine, other causes of seizures must be ruled out. We report the case of a 19 y.o. M who smoked crack cocaine and then ingested an unknown amount to avoid arrest. He had no history of epilepsy but had at least 2 witnessed tonic clonic seizures along with the stigmata of acute cocaine intoxication. He was brought from jail to the emergency department (ED) in what appeared to be a post ictal state. He was afebrile with a heart rate of 110, a blood pressure of 180/70 and respiratory rate of 28/min. He was lethargic and hyperreflexic. After the second tonic clonic seizure in the ED, the patient was given lorazepam 2 mg IV. Blood was drawn for cocaine and metabolite concentrations into tubes containing sodium fluoride at varying time points and immediately frozen. Blood samples were stored frozen and thawed immediately prior to analysis. Cocaine and its metabolites were isolated from blood by protein precipitation with acetonitrile. The supernatant was derivatized to form propyl esters of carboxylic acids, propylation of primary amines, and p-nitrobenzoyl esters of alcohols. The reaction mixture was extracted at alkaline pH with n-butyl chloride and analyzed by GC/MS in selected ion mode. Cocaine concentrations were 0.724, 0.689, 0.279 and 0 $\mu\text{g/ml}$ at 30 minutes before the second seizure and 6, 12, and 24 hours after, respectively. Benzoylcegonine concentrations were 4.569, 4.407, 3.718 and 1.192 $\mu\text{g/ml}$ at 30 minutes before, 6, 12, and 24 hours respectively, after the seizure. Norcocaine concentrations were 0.042 $\mu\text{g/ml}$ 30 minutes prior to the seizure, and 0 at all other time points. Ecgonine methyl ester concentrations at 30 min prior and 6, 12 and 24 hours after the seizure were 1.072, 1.078, 0.527 and 0.244 $\mu\text{g/ml}$ respectively. Blood alcohol concentrations were negative. Evaluation for non-cocaine causes of seizures such as intracranial hemorrhage, cerebral edema, metabolic or electrolyte imbalance and infection were ruled out by CT scan and blood laboratory values. The patient was discharged from the hospital to jail after 27 hours.

#77 Distinguishing Poppy Seed Consumption from Opiate Abuse Using Thebaine as a Marker by Gina Cassella* and Brenda Shaw, Department of Chemistry, University of Connecticut, Storrs, CT 06269 U.S.A., Alan H.B. Wu, Toxicology Laboratory, Hartford Hospital, 80 Seymour St., Hartford, CT 06102 U.S.A., Dennis Hill, Department of Pathobiology, University of Connecticut, Storrs, CT 06269 U.S.A.

Consumption of poppy seeds from various foods may lead to a positive opiate result in urine subjected to drugs of abuse testing. In this study we investigated the use of thebaine, a natural constituent of poppy seeds, as a possible marker for poppy seed use. Thebaine was chosen since it was not found to be a constituent of commercial and illicit drug preparations such as heroin, morphine, or codeine. Poppy seeds were extracted with methanol and analyzed by gas chromatography with an ion trap mass spectrometer (GC/MS) to determine if thebaine was present in the seeds. Spiked urine samples, baseline urine samples (prepoppy seed consumption), and urine samples of poppy seed eaters were analyzed to identify thebaine. Urine samples of documented true opiate (heroin, morphine, codeine) users from a drug compliance program were also investigated to establish that thebaine was not present in these samples.

Urine samples were collected from 10 subjects who consumed between 1-4 poppy seed muffins each containing approximately 11 grams of poppy seeds approximately 6-8 hours after ingestion and during a controlled time interval study. The urine samples were first screened by the Emit II immunoassay for opiates. Positive urine samples were extracted using a copolymeric sorbent utilizing hydrophobic (C-8) and cation exchange (benzenesulfonic acid) phases bonded to silica. The solid phase extracts were analyzed by GC/MS using a probe injection temperature program. Thebaine was detectable in the spiked urine samples and the urine samples of the poppy seed eaters in concentrations ranging from 2 ng/mL to 88 ng/mL. Thebaine was not found in the baseline urine samples, the urine samples of opiate users, or the heroin extracts. Detection of thebaine will be helpful in identifying subjects who are truly positive from illicit opiate use from those who have consumed poppy seed products.

#78 Atypical Opiate Urinary Excretion Pattern by Leon R. Glass^{1,2}, Stephen T. Ingalls^{1,3} and Charles L. Hoppel^{1,3}. ¹Research ²Pathology and Laboratory Medicine Service, 113-B, Veterans Administration Medical Center, 10000 Brecksville Rd., Brecksville OH 44141 U.S.A., ³Case Western Reserve University, Cleveland OH, 44106 U.S.A.

We present two case studies of 43 y.o. apparently identical male twins who displayed an atypical pattern of opiate metabolism. The subjects had a history of opiate abuse and they are currently in a substance abuse treatment program. Treatment included periodic clinical urine drug testing. Urine specimens submitted by these subjects occasionally gave a positive response for opiates by enzyme immunoassay, and these were consequently forwarded for GC-MSD confirmation analysis. The specimens contained as much as 2000 ng/mL of 6-acetylmorphine (6-AM) and less than 350 ng/mL of total morphine.

Heroin is rapidly metabolized in humans to 6-AM, which is further metabolized to morphine and morphine conjugates. Urinary 6-AM is the best diagnostic indicator of heroin abuse. This metabolite, however, is usually present in urine at less than 1% of the concentration of urinary total morphine. We conclude that if testing for 6-AM is performed only when the total morphine is elevated, false negative test results may result.

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