01

URINARY EXCRETION OF BENZOYLECGONINE FOLLOWING INGESTION OF HEALTH TEA

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Four males ingested one cup of Inca Health Tea which contained 1.87 mg of cocaine. Following administration, food and beverages including coffee were ingested ad lib over the course of the study. Urine specimens were collected for 36 hrs post-ingestion. Urine was analyzed for benzoylecgonine (BE) by EMIT-d.a.u., TDx and GC/MS. Creatinine was determined by colorimetry. Positive immunoassay results were obtained for 21-26 hrs post tea ingestion. Discrepant immunoassay results occurred with only one specimen: EMIT, positive; TDx, negative, 0.25 mg/L; GC/MS, 0.273 mg/L. Quantitative TDx results were well correlated with GC/MS results, r^2=0.963, n=45. Maximum, urinary BE concentrations ranged from 1.5-2.8 mg/L, occurring from 4-11 hrs, post ingestion. Total BE excretion in 36 hrs ranged from 1.05 to 1.67 mg, 59% to 97% of the ingested cocaine dose. Urinary excretion rate constants (Km) ranged from 0.037 to 0.045/hr. While the FDA has banned importation of Inca Health Tea, the product may still be available and should be considered when interpreting urinary BE concentrations.

02

VALIDITY ASSESSMENT OF THE ABUSCREEN® ONTRAK™ ASSAY FOR COCAINE **Edward J. Cone*, William D. Darwin and Sandra L. Dickerson, Addiction Research Center, NIDA, Baltimore, MD 21224**

Abuscreen ONTRAK for Cocaine is an easy to use, qualitative test for cocaine metabolite (benzoylecgonine, BE) in human urine. The test kit is self-contained and can be performed in about 3 minutes. The principle of the assay is latex agglutination-inhibition; a negative result is determined by the presence of white particles in the viewing area of the slide, whereas a positive result is indicated by a milky appearance. We performed a validity assessment of ONTRAK by analyzing a set of 290 urines in which BE had been quantitatively determined by GC/MS. The set consisted of drug-free specimens containing known amounts of drug standards and specimens collected from 5 male, volunteer research subjects who provided informed consent and received intravenous doses of cocaine under controlled conditions. Specimens were analyzed in random order under blind conditions and manufacturer's procedures were followed. Two independent test readers recorded results. "Cutoff" readings were assigned when the test was not clearly positive or negative. Concordance of results between ONTRAK and GC/MS for clinical specimens was high and no false positives (<300 ng/mL) were obtained. However, the two readers recorded one and three false negatives (300-1000 ng/mL), respectively, and disagreed on 8 results. Also, there were numerous "cutoff" results in the concentration range of 300-1000 ng/mL. The study indicated that ONTRAK results compared well with GC/MS, but weakly positive specimens (<750 ng/mL) may not produce definitive results in the ONTRAK test.

03

MILENIA--A KINETIC IMMUNOASSAY SYSTEM FOR SEMI-QUANTITATIVE DRUG SCREENING. **C. Hand, D. Burns, R. Smith, A. Moore, H. Wilson, S. El Shami and V. Spiehler*, DPC/ERI, Witney, UK and DPC, Los Angeles, CA 90045**

Milenia is a novel kinetic enzyme immunoassay system in a microplate format which has been applied to drug screening. Patient samples are added to ligand-coated wells, along with ligand-labeled drug antibodies and horseradish peroxidase (HRPO)-labeled drug as tracer. During the initial 30 minute reaction, enzyme-labeled drug competes with drug and drug metabolites in the patient sample for antibody binding sites. During a second 30 minute incubation, the addition of a multivalent anti-ligand creates a bridge between the drug-antibody complexes and the ligand-coated wells. The microplate is then washed to remove unreacted material.

Hydrogen peroxide and o-phenylenediamine are then added, and the rate of color development due to HRPO activity is determined at 450 nm (2,2'-diamino-azobenzene) using a kinetic microplate reader. Reaction rates, measured in milli-optical density units per minute, are inversely related to drug concentrations present in the samples. Intrassay precision for delta-9-THCOOH at 100 ng/ml was 4.2%, interassay precision was 6.2%. Intrassay precision for morphine at 300 ng/ml was 4.9%, interassay precision was 12.9%. Three 96 well microtiter plates can be combined in a batch. Drug quantitation is possible within the range of the calibrators supplied.

04

"REMEDI" IDENTIFICATION OF DRUGS YIELDING FALSE POSITIVE AMPHETAMINE IMMUNOASSAY RESULTS

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Approximately 30,000 urine specimens were screened under NIDA guidelines by the EMIT-d.a.u. Monoclonal Amphetamine (A)/Methamphetamine (MA) Assay (EM). Of these, 54 failed to confirm for Amphetamines by GC/MS. Reassay by the EM, EMIT-d.a.u. polyclonal (EP) and TDx A/MA II assays yielded the following positive results: EM, 54/54; EP, 50/54 and TDx, 8/54. Cross-reacting drugs in these urine specimens were identified by the "REMEDI" Drug Profiling System. The REMEDI is an automated multi-column high pressure liquid chromatographic system. Sample preparation consist of dilution and centrifugation. Following automatic injection, drugs are isolated in two polymeric pre-columns containing PRP-1 and Aminex A-28. Analytical separation is produced in short reversed-phase column coupled to a silica column. Eluted drugs are identified by relative retention time and scanning UV detection. Analysis is completed within 25 min/sample. The spectrum of the unknown is compared to spectral library of over 300 drugs. Immunoassay cross-reacting drugs included phentermine, ephedrine/pseudoephedrine, labetolol, fenfluramine and ranitidine. Identification of these drugs in a single step chromatographic procedure demonstrates the versatility of REMEDI. Reassay of EMIT-d.a.u. positive Amphetamine urines by the TDx A/MA II assay will reduce the number of unconfirmed results for A/MA by GC/MS.

05

RANITIDINE INTERFERENCE WITH THE EMIT-d.a.u. MONOCLONAL AMPHETAMINE/METHAMPHETAMINE ASSAY; REMEDI QUANTITATION OF RANITIDINE IN URINE

Alphonse Poklis*, Karen V. Hall, Jay Still and Steven R. Binder. Dept. of Pathology, Medical College of Virginia, Richmond, 23298-0597, Bio-Rad Laboratories, Hercules, CA. 94547-5340.

The interference of ranitidine with the EMIT-d.a.u. Monoclonal Amphetamine (A)/Methamphetamine (MA) assay (EM) was investigated. Urine specimens collected from 23 patients receiving 150-300 mg of ranitidine daily were analyzed by the EM, EMIT-d.a.u. polyclonal (EP) and TDx A/MA II immunoassays. Quantitative analysis of urinary ranitidine was performed with a BioRad REMEDI Drug Profiling System. The REMEDI is an automated multi-column high pressure chromatographic system which employs on-line sample preparation and utilizes scanning U.V. detection for drug identification and quantitation. Replicate analysis of ranitidine controls during the course of the study yielded the following precision: 3.97 ± 0.05 mg/L (Target, 4.0 mg/L), CV=1.0%, n=3 and 23.4 ± 0.49 mg/L (Target, 25.0 mg/L), CV=2.1% (n=3). The recovery determined by standard addition yielded; 90.5 ± 12.5%, n=10. Patient urines contained 7-271 mg/L ranitidine. Only patient specimens and urine with ranitidine added at concentrations greater than 91 mg/L gave false positive EM results, 12/63 patient urines. All false positive EM results occurred in the first or second void after ingestion. No false positives occurred with the EP or TDx A/MA II assays. While ranitidine may cause false positive EM results, we found the incidence of this effect in day to day drug testing is very low.

06

FLUOXETINE ON MEDICAL EXAMINER CASES USING GC/ITD

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Concentrations of the new widely used antidepressant drug Fluoxetine and its desmethyl metabolite were determined in autopsy samples using a Perkin Elmer GC/ITD. Isolation was performed by a liquid/liquid extraction. GC/ITD conditions include a 15 meter 0.320 Restek RTX1 column: oven temperature programming and ITD full scanning from 40-400 amu.

Medical Examiner cases involving fluoxetine will be evaluated. In one multiple drug overdose, high concentrations of fluoxetine were found in blood, liver and urine. The values will be compared with those by other techniques such as HPLC.

07

SCREENING AND QUANTITATION OF BENZODIAZEPINES IN URINE/BLOOD WITH HPLC

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Benzodiazepines are extracted from 2mL of urine or blood with 4mL of toluene:hexane:iso-amyl alcohol (78:20:2; v/v/v). The residues of the organic phase are reconstituted in 50uL of methanol. 5uL of the sample is injected into an C18 column with a mobile phase of methanol:H_2O (3:1; v/v). Flow rate is 1mL/min isocratic. The identification of benzodiazepines is based on RRT of analyte at 240nm and its UV spectrum between 200-400nm obtained from the diode-array detector. Quantitation is by comparison peak area of analyte to that of internal standard (prazepam).

The HPLC data demonstrates that the linearity of the standard curves ranged from 0.02mg/L to 1.50mg/L (r=0.9984-0.9997). The RRT of some benzodiazepines tested are listed below.

	RRT		RRT
Chlordiazepoxide	0.58	Alprazolam	0.82
Lorazepam	0.67	nor-Diazepam	0.88
Oxazepam	0.73	Diazepam	1.00
Temazepam	0.79	Prazepam	1.53

In conclusion, this rapid extraction procedure and HPLC method are simple and sensitive for analysis of benzodiazepines. No buffer is required for the mobile phase.

08

THE DETERMINATION OF PANCURONIUM BROMIDE (PAVULON) FROM EMBALMED TISSUES

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A hospital nurse was suspected of administering an unauthorized injection of a neuromuscular blocking agent. Toxicologic analysis of the patient's urine proved inconclusive due to a delay, app. 48 hrs., in sample collection. Subsequent hospital and police investigations into patient code rates and hospital drug inventories indicated that numerous deaths might have been attributable to pancuronium poisoning.

In total, 33 bodies were exhumed. Liver, kidney and lung from each case was initially screened fluorometrically for pancuronium using Rose Bengal as the pairing agent. All presumptive positive findings were then submitted to GC/MS for confirmation. This was accomplished by pairing the drug with bromophenol blue, extracting the complex and submitting the extract to thin layer chromatography, followed by elution at the appropriate R_f, reextraction by pairing with KI, and finally, GC/MS analysis in the SIM mode.

Pancuronium appears to undergo pyrolytic N-demethylation in the injection port to yield a tertiary amine which is amenable to capillary GC analysis. The mass spectrum of this compound yields a base peak at m/z 322 with secondary peaks at m/z 292, 323, 338, 396 and 397.

Pancuronium was confirmed to be present in eight of the exhumed bodies. This finding was instrumental in the subsequent prosecution and conviction of the accused.

09

EVALUATION OF RAPID ASSAYS FOR SERUM THEOPHYLLINE: On-Site Testing in the Emergency Medical Department

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As a quality assurance study, we evaluated the rapid on-site theophylline assays by the AccuLevel (Syva,) and the Vision (Abbott) methodology and compared the results to the reference method of High Performance Liquid Chromatography (HPLC). Quantitative serum theophylline results were needed in order to provide timely treatment for patients presenting to the Emergency Medical Department (EMD).

A good correlation between the theophylline concentrations simultaneously determined by AccuLevel and HPLC was observed; r^2 =0.916, n=96; and between Vision and HPLC, r^2 = 0.947, n=93.

A slight positive bias by both AccuLevel and Vision was noted with the mean of concentrations being 11.7 mg/L and 13.2 mg/L for HPLC and AccuLevel, respectively. Precision of the assay was found to be 4.6% (n=10) and 4.5% (n=21) for AccuLevel and Vision respectively. Because of the delay in transporting specimens from the EMD to the Clinical Laboratories at our Medical Center, the EMD was able to obtain quantitative theophylline serum values from 30 minutes to 1.5 hours sooner by using the AccuLevel or Vision, on-site test.

10

DEATH DUE TO TOLUENE ABUSE: A Case Report

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A 39 year old male with a known history of drug abuse was found dead with a rag and paint bucket over his face.

Routine drug screening following autopsy revealed the presence of the following: amitriptyline, nortriptyline, hydrocodone, diazepam, desmethyldiazepam, cocaine, benzoylecgonine, butalbital, acetaminophen, acetone, isopropanol and toluene. Toluene analyses were determined by HSGC-FID on a PE F 45-GC 6' carbopack B, 5% carbowax column. Concentrations were in mg/L or mg/kg. Blood:34, Liver:73, Brain:182, Lung:13, Gastric:11, Vitreous Humor:<1, Urine:<1. All drugs and metabolites were at low or therapeutic concentrations.

This poster will show the method and results for toluene in post autopsy tissue specimens.

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FATALITY ASSOCIATED WITH ENFLURANE AND BUTORPHANOL

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A 66 year old latin male was seen in a Miami, Fl., surgical center for a facelift. Following surgery Stadol (butorphanol) was given for pain. The patient suffered a cardiopulmonary arrest and died. The clinic maintained only local lidocaine anesthesia and butorphanol were administered.

Standard toxicology analysis revealed thiopental, pentobarbital and benzodiazepines detected in the urine. Heart blood analysis found thiopental (trace), pentobarbital (trace), diazepam (0.04mg/L), nordiazepam (0.034mg/L) and lidocaine (1.74 mg/L). An unknown peak on the heart blood volatiles screen was identified as enflurane by headspace GC/MS. Quantitative analysis of this analyte by GLC/ECD using methoxyflurane internal standard found 32.0 mg/L in the heart blood. A butorphanol level of 0.006 mg/L was measured in the heart blood by multiple ion monitoring GC/MS using codeine as internal standard and a heptafluorobutyryl derivative. The analysis demonstrated not only the presence of a local anesthetic agent, lidocaine, but general anesthetics as well. All the analytes were in therapeutic and subtherapeutic concentrations with the exception of butorphanol, which was approximately three times therapeutic. At autopsy no anatomic cause was found to explain the sudden death. The case was signed out as Cause of Death: hypoxic encephalopathy due to administration of anesthesia. To our knowledge this is the first reported death case associated with butorphanol. This case is currently under investigation by DPR and the State Attorney's office.

12

PRELIMINARY EVALUATION OF THE ABBOTT TDx FOR SCREENING OF d-METHAMPHETAMINE IN WHOLE BLOOD SPECIMENS.

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The Abbott TDx Amphetamine/ Methamphetamine II immunoassay was applied to the determination of d-methamphetamine in whole blood. The TDx assay was found to be specific showing a 100% cross reactivity with d-methamphetamine and only 5% cross reactivity with the l-methamphetamine isomer. Whole blood was fortified with d-methamphetamine at concentrations ranging from 25 to 1,000 ng/mL. Sample preparation techniques for the whole blood matrix was evaluated by analyzing whole blood calibrators direct, diluted with buffer, urine calibrators diluted with whole blood, and precipitated whole blood calibrators using zinc chloride, trichloroacetic acid, sulfanilic acid, and methanol precipitation techniques.

The whole blood calibration curves showed a linear range of 50 to 400 ng/mL and a sensitivity of 25 ng/mL using 200 uL of blood with protein precipitations. The within-run precision for d-methamphetamine at concentrations of 60 and 200 ng/mL showed a CV% of 8.5 and 4.9 respectively. The Abbot TDx results were compared to GC-MS quantification of amphetamine and methamphetamine for 12 positive case specimens.

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APPLICATION OF THE TECHNICON CHEM I CAPSULE CHEMISTRY SYSTEM TO SYVA EMIT DRUGS OF ABUSE ASSAYS: A RAPID AND COST EFFECTIVE APPROACH TO SUBSTANCE ABUSE TESTING.

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The Technicon Chem I system incorporates the versatility of a discrete random access analyzer with the throughput of a continual flow analyzer. By utilizing 7 ul of a 1:7 dilution, the Chem I offers a very cost effective approach for high volume screening of urine specimens for drugs subject to abuse.

Eleven EMIT DAU and two EMIT 700 Series (Syva Corporation) were examined for precision and performance accuracy on the Chem I. Negative Controls (N=90), Low Controls (N=135) and Medium Controls (N=120), were run over a thirty day period, (N=35). The within-run precision ranged between 0.2 to 1.4% for all assays and all levels, mean 0.5%. The within day precision ranged from 0.2 to 1.6% for all assays and all levels, mean 0.6%, while the day to day presicion ranged from 0.2 to 5.2% for all assays, all levels.

One hundred and eight urine specimens that had been previously analyzed using Technicon RA 1000 and Technicon RA-XT analyzers were run on the CHEM I. The CHEM I compared well to these systems, with a qualitative correlation that was greater than 99%.

This data suggests that the CHEM I offers a rapid, accurate and cost effective alternative for medium to high volume urine drug screening.

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COMPARATIVE STUDY OF THE RCCHE ABUSCREEN ONTRAK SYSTEM

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A comparative study was conducted to ascertain the accuracy and reliability of the Roche, Abuscreen ONTRAK system for the screening of urine samples for drugs of abuse. The study was in response to questions raised by Colorado correctional facility personnel who were using the ONTRAK system on a routine basis for client screening.

Four separate assay kits were provided by Roche Diagnostic Systems. They were the THC, cocaine, amphetamine, and morphine. Each kit was used according to the manufactures instructions.

Samples for the study were obtained from the normal daily specimen load received by the Colorado Department of Health. After screening and confirmation had been completed, forty specimens were then selected for analysis by each ONTRAK assay with a distribution of positives and negatives of approximate equal numbers.

The results obtained showed that the Roche ONTRAK can be used as an effective tool for the on site screening of urines in correctional facility settings. However, the caveat being that all positives then must be screened and confirmed in a forensic laboratory by more traditional methods.

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EVALUATION OF ADx® & TDx® SERUM BARBITURATE ASSAY. A. M. Hoffman,* D. M. Andrenyak and D. E. Moody, Center for Human Toxicology, Univ. of Utah, SLC, UT 84108.

Abbott ADx and TDx (Xsys) instruments were evaluated for serum barbiturate detection using recently developed reagents. Secobarbital (seco) calibrations (0 to 40 µg/ml) were stable for at least 48 days. For TDx precision seco controls at 3.0, 7.5, & 30.0 µg/ml, and a multiconstituent (MCC) control (seco-3.0; acetaminophen (Acet)-15.0, & tricyclic antidepressant (TCA)-100 ng/ml) were measured in replicates of 5 over 10 days. Between-run (BR), within-run (WR), & total (T) cvs ranged from 2.1-4.2, 2.1-3.3, & 2.9-5.1%. For ADx, the 3 seco controls were run alternately in batch and combination modes; the latter included TCA & Acet controls in each run. Medium & high controls were significantly lower in the combination mode (95.7 & 97.1% of batch). Combined BR, WR, & T cvs ranged from 1.1-2.8, 2.6-6.3, & 2.6- 6.3%. No difference was found in barb content of the MCC control run in panel vs combination mode on ADx, with BR, WR, & T cvs from 1.3-2.6, 3.0-3.3, & 3.3-3.6%. Accuracy was evaluated by comparison with EMIT and GC/MS assays. Cross-over assays for each method were within 10% of target. ADx, TDx and EMIT assays had respective cross-reactivities (at 3.0 µg/ml) for amobarbital (50, 55 & 62%), butalbital (70, 78 & 74%), pentobarbital (58, 72 & 74%), and phenobarbital (36, 48 & 48%). Of 101 "negative" serum specimens, 3 assayed > cutoff, 2 µg/ml, by both ADx & TDx, and were confirmed to contain > 15 µg/ml phenobarbital by GC/MS. Of the 2 assayed by EMIT, 1 was above and 1 below its 3 µg/ml cutoff. The LOQs (mean ± 3SD for the negative serums) were 0.64 & 1.28 µg/ml for ADx & TDx. Of 90 "positive" specimens: 17 were negative and 48 positive by all methods (GC/MS cutoff, 2 µg/ml); 16 (5 with all assay cutoffs at 3 µg/ml) were positive by Xsys & GC/MS, but EMIT negative: 8 negative by 1, or both, Xsys and EMIT, but GC/MS positive; and 1 positive by Xsys, but negative by EMIT and GC/MS (1.3 µg/ml butalbital). In conclusion, the Xsys FPIA offers comparable accurracy and greater sensitivity for serum barbiturate immunoassay analysis.

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EVALUATION OF ADx® & TDx® URINE BARBITURATE II ASSAY. D. M. Andrenyak,* A. M. Hoffman and D. E. Moody, Center for Human Toxicology, Univ. of Utah, SLC, UT 84108.

Abbott ADx and TDx (Xsys) instruments were evaluated for urine barbiturate detection using recently developed (Barb II U) reagents. Secobarbital (Seco) calibrations (0 to 2000 ng/ml) were stable for at least 16 days. For TDx precision Seco controls at 300, 800, & 1500 ng/ml, and a multiconstituent (MCC) control (Seco-300; carboxy-THC (Can)-50, & amphetamine (AM)-100 ng/ml) were measured in replicates of 5 over 10 days. Between-run (BR), within-run (WR), & total (T) cvs ranged from 3.0-5.6, 2.3-3.8, & 3.0-5.9%. For ADx, the 3 Seco controls were run alternately in batch and combination modes; the latter included Can & AM controls in each run. No significant differences occurred between batch and combination modes. Combined BR, WR, & T cvs ranged from 3.2-8.7, 2.6-5.8, & 3.5-9.2%. No difference was found in barb content of the MCC control run in panel vs combination mode on ADx, with BR, WR, & T cvs from 5.5-6.8, 5.2-7.0, & 5.5-7.2%. Accuracy was evaluated by comparison with Xsys I, RIA, EMIT and GC/MS assays. Cross-over assays for each method were within 10% of target, except for Xsys I analysis of RIA stds (at 16%) and RIA analysis of Xsys stds, at 22%. Xsys II, Xsys I, RIA and EMIT assays had respective cross-reactivities (at 700 ng/ml) for amobarbital (33, 105, 14 & 47%), butalbital (115, 114, 21 & 56%), pentobarbital (69, 68, 26 & 64%), and phenobarbital (PhB) (63, 132, 10 & 42%). Of 100 "negative" urine specimens, none screened positive by any of the immunoassays. The LOQs (mean ± 3SD for the negative serums) were 245 (Xsys I), 56 (Xsys II), 28 (RIA) & 128 ng/ml (EMIT). Of 99 "positive" specimens: 5 were negative and 79 positive by all methods (GC/MS cutoff, 200 ng/ml); 12 were positive by all methods except RIA; 1 was positive by Xsys II and GC/MS only (476 ng PhB/ml); 1 was positive by ADx II and GC/MS only (202 ng PhB/ml); and 1 positive by GC/MS only (207 ng PhB/ml). In conclusion, the Xsys Barb II U assay offers comparable accurracy and improved sensitivity for urine barbiturate immunoassay analysis.

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EVALUATION OF THE ABBOTT TDx AND ADx SERUM BENZODIAZEPINE ASSAYS ON POSTMORTEM BLOOD

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FPIA analysis of 159 postmortem specimens was performed after centrifugation to remove cellular debris and clots. Of 60 blood specimens found to contain classical and low-dose benzodiazepines by GC/ECD, GC/MS or HPLC, all but two were "positive" (>12 ng/ml) by TDx and all but one by ADx; each of these two contained 13 ng/ml alprazolam by GC/ECD.

Of 49 blood specimens originally screened as "negative" by chromatography, 43 were negative by TDx and ADx (<12 ng/ml). The remaining 6 specimens were re-analyzed by chromatography after obtaining detailed case histories: all were subsequently found to contain benzodiazepines; 5/6 had less than 50 ng/ml.

Of 50 randomly selected blood specimens 38/50 were negative and 11/50 were positive for benzodiazepines by both chromatography and TDx/ADx. The remaining blood specimen could not be confirmed by chromatography, although a trace of oxazepam was detected in urine from the same person.

We conclude that the Abbott FPIA assay is valuable for the detection of benzodiazepines in postmortem blood. However, confirmation of presumptive FPIA positive results <100 ng/ml may require substantial analytical effort in some cases.

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URINARY TRICYCLIC ANTIDEPRESSANT SCREENING: COMPARISON OF RESULTS OBTAINED WITH ABBOTT'S ADx AND SYVA's ETS SYSTEMS

Gerard H. Keenan,* Susan Barlotta, and Michael Lehrer (Department of Toxicology, Long Island Jewish Medical Center, New Hyde Park, NY 11042).

The need for rapid tricyclic antidepressant (TCA) screening of human urine has long existed in emergency overdose situations. More recently, human urine TCA screening was recommended by the NYC Department of Health for laboratories engaged in forensic drugs of abuse testing with the implication that this may become a mandated requirement by NYC. Our study was designed to ascertain the technical suitability of urine TCA screening by immunoassay techniques and to provide a preliminary assessment of sensitivity and specificity of such screening methodologies.

Human urine was tested for the presence of TCA's with the Abbott ADx TCA assay and on the Syva ETS with the SYVA Emit tox serum TCA assay. The ETS system was adapted to analyze TCA samples. A 300 ng/ml nortriptyline calibrator cutoff was used with the ETS system and a 75 ng/ml imipramine calibrator cutoff with the ADX system. Negative urine was spiked with a variety of TCA's and with phenothiazine medications that have potential for yielding false positive results. Verification of samples was performed by thin layer chromatography and/or gas chromatography/mass spectrometry. The different compounds were added to urine at levels of 200, 400 and 1,000 ng/mL. At 1,000 ng/mL, all compounds tested (including the non-tricyclic medications) gave positive TCA results on the ETS. Similar results were obtained with the ADX system where all compounds with the exception of thioridazine gave positive TCA results. At the 400 ng/mL level, all TCA's tested and some of the non-tricyclic medications gave positive ETS and ADx responses. At the 200 ng/mL spike, all spiked compounds gave negative ETS results. In contrast, all TCA's gave positive ADx results. In addition, some of the non-tricyclic medications also gave positive ADx results when spiked at the 200 ng/mL level.

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THE ANALYSIS OF SPLEEN SPECIMENS FOR CARBON MONOXIDE.

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Crucial to the investigation of aircraft fatalites is the analysis of biological specimens for carbon monoxide (CO). In many cases, blood specimens are unavailable or unsuitable for analysis, and the testing of an alternate specimen for CO becomes necessary. Spleen specimens provide a rich source of red blood cells, hence can be a primary substitute for blood. To verify this, 40 paired blood and spleen specimens were analyzed for CO using a gas chromatographic method. Ten specimens with a spleen CO saturation level (sat.) of less than 10% were associated with corresponding blood specimens with a CO sat. less than 10%. Fifteen of the 18 spleen specimens with CO sat. greater than 29% were associated with blood specimens with greater than 48% sat. Results were inconclusive when the spleen CO sat. was between 10 and 29%. We concluded that spleen CO sat. can reflect blood CO sat. in certain situations.

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d_6-11-NOR-Δ^9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID AS A NEW INTERNAL STANDARD FOR THE GC/MS ANALYSIS OF THC ACID METABOLITE IN BIOLOGICAL SPECIMENS

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GC/MS analysis of biological specimens is believed to be the most forensically accepted method for confirming the presence of abused drugs. 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) is the major metabolite of tetrahydrocannabinol (THC) for which all testing (including GC/MS) is directed as indication of marijuana use. The currently available internal standard for THC-COOH is d_3-THC-COOH where the deuterium is located in the side chain. In addition to the high cost of this compound, it suffers from the disadvantage of a limited dynamic range of analysis when the methyl derivative is used. This is because of a contribution to one of the internal standard ions (m/z 316) from a fragmentation of the natural drug which involves loss of the side chain. The new internal standard avoids these disadvantages. The six deuterium atoms are located in the two methyl groups on Carbon 6 of the dibenzopyram structure. The dynamic range of analysis using the new internal standard as well as correlation data with the d_3-THC-COOH will be presented.

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STABILITY STUDY OF THE DEUTERATED INTERNAL STANDARDS

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Stable isotope labeled compounds are used with increasing frequency by drug testing laboratories as internal standards for confirmation and quantitation of drugs of abuse. Radian Corporation has been supplying deuterated internal standards to the drug testing community for several years. The stability of these standards over time has been a concern to both Radian Corporation and users of these standards. We undertook a study using HPLC and GC/MS (SIM mode) to establish the stability of standards prepared over a two-year period. Chemical degradation of the compound was determined by comparison of the HPLC response of a freshly prepared standard with that of archived samples from previous lots. Isotopic stability was determined on the same samples by using GC/MS (SIM mode) to compare the ion abundance ratios of the labeled compound and any degradation products. Our study indicated that these standards are chemically and isotopically stable over the study period when stored at -10°C.

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DISPOSITION OF LEAD IN THE TISSUES OF FISH FROM AN ACIDIFIED LAKE.

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Lead concentrations were measured in various tissues of Perca flavescens (yellow perch) and Catostomus commersoni (white suckers) collected from an acidified lake in the Adirondack Mountain Acid Rain Belt, Darts Lake, which is known to have elevated lead concentrations in the sediment and water, was chosen for this study. Lead concentrations in the muscle, liver, kidney and bone were compared with those of the surrounding habitat including: the water, sediment and potential food sources of the fish. This was done in order to assess the tissue distribution and potential bioaccumulation of this toxic element. Lead concentrations were found to be the highest in the bone of both species of fish followed by the liver, kidney and muscle. Lead bioaccumulation was also shown to occur. Our results indicate that elevated levels of lead in the surface sediments are not reflected in the tissues. However, lead biomagnification was demonstrated for some of the potential foods as well as the water.

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TRACKING THE SOURCE OF A GASOLINE STORAGE TANK LEAK.

J. H. Bidanset*, C. J. Abraham, J. Mannetta and W. C. Long.

Leaking underground gasoline storage tanks have produced a number of environmental and toxicologic problems with significant forensic implications. In the case under consideration, an underground gasoline storage tank leak resulted in the saturation of soil, the contamination of an underground aquifer, the leaking of virtually pure gasoline into the basement of a food corporation (A sump pump removed the gasoline to an above-the-ground storage tank.) and a toxic concentration of various hydrocarbons in the basement and other work areas of the building. A brief review of the toxicity of the components of gasoline will be presented. Sampling and analysis were in accordance with approved NIOSH methods. Organic vapors in air samples were absorbed on activated charcoal, desorbed with carbon disulfide and analyzed by gas chromatography. Concentrations in air of aliphatic and aromatic hydrocarbons were determined in the basement and the first floor production area. Virtually raw gasoline samples obtained from the sump were compared to samples of gasoline obtained from local service stations. Based on a comparison of the gas chromatograms, the source of the leak was determined with reasonable scientific certainty.

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THE EFFECTS OF EMBALMING OF TISSUE ON THE STABILITY OF DRUGS.

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Sprague Dawley rats were dosed intravenously with phenobarbital, amitriptyline and morphine. Allowing a suitable period for distribution of the drugs, the animals were then sacrificed using carbon dioxide. The animals were then embalmed with commercially available embalming fluid. Liver, brain, kidney and heart were obtained by necropsy. The stability of the drugs were then determined by quantitating (GC/MS) at various times up to eight weeks after embalming.

Phenobarbital remained stable in all four tissues during the entire duration of the study.

Amitriptyline showed losses of greater than 40% in kidney and heart. Nortriptyline was unstable in the presence of embalming fluid. Nortriptyline was methylated to amitriptyline, a reaction which was dependent upon pH and formaldehyde content.

Morphine demonstrated greater than 50% losses in liver, brain, and kidney; greater than 80% losses in heart.

Stability appears to be dependent upon the lipophilicity of the drug, the pH of the tissue, and the amount of formaldehyde which perfused the particular tissue site.

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SIMULTANEOUS ASSAY FOR COCAINE (C), BENZOYLECGONINE (BE), ECGONINE METHYL ESTER (EME), NORCOCAINE (NC) AND COCAETHYLENE (CE) IN HUMAN BIOFLUIDS BY GC/MS. **William D. Darwin*, Thomas E. Maguire and Edward J. Cone, Addiction Research Center; NIDA, Baltimore, Md 21224.**

Cocaine is extensively metabolized in man to BE, EME and NC. CE has been identified in biofluids when cocaine is taken in conjuction with alcohol. The simultaneous assay for all cocaine components has been difficult in the past due to inefficient isolation techniques. We developed a simultaneous assay for cocaine and its metabolites that can be used with a variety of human biofluids. The 130-mg CLEAN-SCREEN® DAU solid phase extraction column was used to extract C, BE, EME, NC and CE from biofluids and tissue, i.e., liver. The extraction conditions were as follows: pre-filter the sample through a 40-µm filter; add deuterated internal standards and buffer with sodium acetate buffer (pH 4.0, 2.0 N); condition the columns with methanol and water; after the samples were introduced to the column, wash with water, 0.1 N hydrochloric acid and methanol; and elution was accomplished with methylene chloride:2-propanol (8:2) with 2% ammonium hydroxide. The extracts were concentrated under a nitrogen stream, derivatized with BSTFA (with 1% TMCS) and analyzed by GC/MS in the SIM mode. Extraction efficiencies were essentially quantitative for all components with the exception of EME which was ~65%. Correlation coefficients of the standard curves were ≥0.98. Responses were linear across a concentration range of 25-1000 ng of drugs/ml in biofluid with the lower limit of sensitivity estimated at 2.5 ng/ml. This assay is applicable to pharmacokinetic studies of cocaine and its metabolites in urine, blood, plasma and saliva.

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FULL SPECTRA GC/MS IDENTIFICATION OF DELTA-9-CARBOXY-THC IN URINE WITH THE FINNIGAN ITS40
*Gary H. Wimbish, Kent G. Johnson

The dimethyl derivative of delta-9-carboxy-THC can be identified and quantitated with full spectra identification via the Finnigan ITS40. The limit of detection (LOD) and limit of quantitation (LOQ) are 2.5 ng/ml and 5.0 ng/ml, respectively. Delta-8-carboxy-THC-d_6 is used as the internal standard since it allows chromatographic base line resolution from delta-9-carboxy-THC; thus, the full spectra of delta-9-carboxy-THC can be achieved throughout the dynamic range of 5 ng/ml to 1000 ng/ml. Auto-quantitation software locates, identifies and quantitates delta-9-carboxy-THC and its internal standard by using a designated retention time window and 16 of the most significant masses for delta-9-carboxy-THC and its internal standard. Experimental data reveals that a fit value of 900 provides adequate intensity of the analyte for quantitation with a signal to noise ratio of 10:1 while assuring quality full spectra identification. A fit value of 800 is required to assure detection via a signal to noise ratio of 3:1 with a quantity full spectra match while blank values for fit were less than 100. The full spectra correlation of 900 from the comparison of the analyte to library suggests a higher statistical significance for drug identification compared to the ion ratioing techniques. This method may eliminate the phenomenon of the three ion ratio "fall out" that is prevalent during routine quadrupole SIM data acquisition.

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QUINIDINE INHIBITS *IN VIVO* METABOLISM OF AMPHETAMINE IN RATS : IMPACT UPON CORRELATION BETWEEN GC/MS AND IMMUNOASSAY FINDINGS IN URINE.
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Amphetamine (Amph) is metabolized by cytochrome P-450 (P450) to *p*-hydroxyamphetamine (pHA) and phenylacetone. P450 modulation by genetic polymorphisms and xenobiotic interactions is isozyme-specific. Little is known concerning the isozyme selectivity of Amph metabolism. Quinidine, a selective inhibitor of an isozyme (P450db) which displays genetic polymorphism, was tested as an inhibitor of the metabolism of Amph to pHA *in vivo* .

At 0 hr rats received (po): no treatment (I), 80 mg quinidine / kg in 50% ethanol (II), or 50% ethanol (III), followed at 2 hr by 15 mg d-Amph sulfate / kg (po). Urine was collected at 0, 24 and 48 hrs. Amph and pHA concentrations were determined by GC/MS. The ethanol control (III) had no significant effect on Amph metabolism. Quinidine pretreatment (II) resulted in a significant decrease in the excretion of pHA at 24 hr to 7.2% and at 48 hr to 24.1% of the vehicle-control levels, accompanied by a significant increase in Amph excretion between 24 and 48 hr to 542% of the control. Quinidine's inhibition of Amph metabolism, suggests that Amph metabolism may, in part, be mediated by an isozyme of P450 which displays genetic polymorphism.

The inhibition of Amph metabolism results in an increased ratio of parent drug to metabolite (metabolic ratio) in the urine, which mimics the effect of genetic polymorphisms. As metabolites may contribute to the immunoreactive response in immunoassays, this alteration in metabolic ratio has the potential of affecting their results. Cross- reactivity studies demonstrated that pHA is recognized by commercial Amph immunoassays, to a greater degree than the metabolites benzoic acid and phenylacetone. Rat urine with the quinidine-induced increase in metabolic ratio was associated with a decrease in the percentage of non-comfirmed immunoassay results. Genetic polymorphisms or drug interactions may therefore affect the number of samples identified as presumptive positives.

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A GC/MS ASSAY FOR AMPHETAMINE AND METHAMPHETAMINE WITH STEREOISOMER SEPARATION AND REDUCED INTERFERENCES
by Robert O. Hughes, William E. Bronner and Michael L. Smith,* Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Washington, DC 20306-6000

A gas chromatography/mass spectrometry assay was developed for the detection of amphetamine and methamphetamine in urine. These drugs were detected as carbamate derivatives following reaction with (-)-menthyl chloroformate. Selected ion monitoring of the 226, 182 and 227 dalton ions for amphetamine and the 240, 102, and 196 dalton ions for methamphetamine provided positive analyte identification with detection limits less than 20 nanograms per milliliter (ng/mL). Instrument response was linear beyond 6000 ng/mL. The intra-assay coefficient of variation was 5% using deuterated internal standards. Chromatographic separation of over-the-counter l-methamphetamine from illicit d-methamphetamine was accomplished on an achiral DB-5 column. No interferences were observed from ephedrine, pseudoephedrine, phenylpropanolamine or 2-phenylethylamine.

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1990 SOFT ABSTRACT

Barbiturates: Chemical Ionization Compliments electron Impact Ionization GC/MS for their accurate Identification.

Proper identification of barbiturates is challenging because a number of barbituric acid analogs are structural isomers of one another. As an instrumental technique, GC/MS offers the greatest opportunity accurate barbiturate identification. Yet, in many cases, identification between isomeric barbiturates is based solely on retention time data since selected ion monitoring techniques limit the confidence in a mass spectral identification. Even other structural specific instrumental techniques such as IR spectroscopy fall short in judicious barb differentiation due limited divergence of wave numbers. Major advances in ion trap mass spectrometry give a new meaning to highly sensitive full scan EI and CI identification of Barbiturate drugs in biological extracts.

The data shown in this paper demonstrates that the isomeric barbiturates can be clearly detected, identified and quantitated at concentrations down to 10 ng/mL in urine. The use of isobutane chemical ionization trivializes the proper identification of barbiturates in biological samples. The quantitative linear dynamic range for the barbiturate assays is over 1.5 orders of magnitude. Reproducible identification of the targeted barbs by full scan EI complimented by CI demonstrate the need for these GC/MS techniques for proper barb identification. Solid phase extraction of drugs permit high recoveries and clean biological extracts.

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INTERVAL TIME OF DEATH DETERMINATION
IN ENZYME MODIFIED VITREOUS FLUID

Vitreous Potassium levels have been used by Forensic Pathologists to determine the interval time of death. The analysis of Potassium levels have, in general, been the burden for the toxicology laboratory. Wide variation of Potassium levels have been attributed to poor sampling, variation from eye to eye and extreme variation in temperature and the conditions to which the deceased has been exposed during the post mortem interval.

The vitreous humor is viscous and often gelatinous in nature. Because of this viscosity, dilution done either manually or automatically is difficult and inaccurate. A unique method whereby the vitreous matrix is enzymatically destroyed will be presented. The resultant, clear liquid was analyzed both by specific ion electrode and by flame photometry. Results by the both methods correlated extremely well, and the coefficient of variation (CV's) decreased from 1.4 to .625 post enzymatic treatment. A correlation by the post mortem interval time of death and Potassium levels in vitreous fluid which has been enzymatically digested will be presented.

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A FORENSIC MARKER FOR A GENETIC DISEASE OFTEN MISDIAGNOSED AS SUDDEN INFANT DEATH SYNDROME. Philip Kemp, B.S. Texas College of Osteopathic Medicine, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107. Southwestern Institute of Forensic Sciences, 5230 Medical Center Drive, Dallas, Texas 75235.

Sudden Infant Death (SIDS) has been associated with medium chain acyl-coA dehydrogenase (MCAD) deficiency, an inborn error of fatty acid oxidation.

A large cohort of SIDS victims were analyzed for the presence of dodecanoic acid (C12) by gas chromatography. A subgroup (7-8%) of these cases had a significantly higher blood concentration than age-matched controls, suggesting MCAD deficiency.

An animal study using Sprague-Dawley rats was done to mimic the effects of MCAD deficiency. Increased blood concentrations of dodecanoic acid were seen as well as the sudden death of a significant number of the animals.

The data indicate that dodecanoic acid is a blood marker for MCAD deficiency, a genetic disease associated with Sudden Infant Death Syndrome.

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LINEAR LEAST SQUARES STANDARD CURVE CALCULATIONS OF MASS SPECTRAL DATA.

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METHOD: A computer program (macro in pseudo-pascal Hewlett-Packard, H/P) for use on the data from the GC/MS for the accurate automated data processing of mass spectral data by linear least squares. Standards and blank mass spectral data are reduced to acquire the peak area for each analyte including the internal standard. The response, peak area divided by internal standard area, are calculated for each analyte and the responses stored. The individual responses are statically reduced by linear least squares to acquire the best fit to the data. The slope and intercept of the standard curve along with the standard error of the estimate and coefficient of correlation are printed with each analytical run. The program uses two or more standards and blank as required. The high standard is near the upper limit of linearity. The low standard is near or 20 % below the administrative cut off point.

DATA: Examples of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, amphetamine, methamphetamine, codeine, morphine, phencyclidine, cocaine, and benzoylecgonine are presented with their associated standard curves. The response for each standard and blank is calculated. The responses are then reduced by linear least squares program to generate the best fit standard curve. The intercept corrects for the variation in blank readings. The slope corrects for minor variation in the response of each of the standards. Each sample response is then reduced by the effect of blank response (intercept) followed by calculation of its concentration from the best fit (slope) of the standard curve.

CONCLUSION: The use of statical reduction of the standard curve by linear least squares with each analytical set of unknowns removes much of the variation with in run and from run to run and increases the accuracy of the unknowns. The factors generated are printed and reviewed prior to calculating the individual controls and samples. The time required to generate the additional information is minimal. The increased information with in a run is useful in detection and resolution of errors. This program is an additional aid to the toxicologist in the mass spectral data reduction process.

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PLASMA AND SALIVA THC LEVELS AND BEHAVIORAL EFFECTS FOLLOWING REPEATED MARIJUANA SMOKING **M.A.Huestis*, S.J.Heishman and E.J.Cone, Addiction Research Center, NIDA, Baltimore,MD., 21224**

In social settings, marijuana may be smoked over prolonged periods of time resulting in substantial intake of Δ9-tetra-hydrocannabinol (THC). We monitorad plasma and saliva THC levels and behavioral effects in male subjects following repeated marijuana smoking to determine the relationship between drug levels in biofluids and drug-induced effects and to determine if acute tolerance could be demonstrated. Three subjects smoked 1, 2 or 4 active marijuana cigarettes; subjects smoked two cigarettes containing either 0.0 or 2.8% THC in the AM and two more cigarettes four hours later in this randomized, placebo controlled, double blind study. Psychomotor and cognitive taska were administered on days 1 and 2 to evaluate performance effects. Subjects were domiciled on a closed research ward. Plasma and saliva levels had peaked by the first 30 minute sample and demonstrated a dose-effect response over the four drug conditions. Repeat dosing in the AM and PM resulted in similar plasma THC levels, whereas saliva levels were more variable. Saliva and plasma THC levels decreased rapidly but remained detectable for 7 hrs following one active marijuana cigarette and up to 24 or 31 hrs, respectively, following smoking of 2 or more active cigarettes. Plasma and saliva THC levels covaried temporally with subjective and performance effects. The digit recall task was the most sensitive indicator of marijuana-induced impairment. Increasing dose led to diminished accuracy. Acute tolerance was not demonstrated to the pharmacologic effects of marijuana.

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DRIVING UNDER THE INFLUENCE OF DRUGS (DUID) IN VIRGINIA: 1988-1989. P.J. Soine*, PH.D. AND J.C. Valentour, PH.D. Division of Forensic Science, PO BOX 999, Richmond, Virginia 23208.

Between April, 1988, when new DUID laws took effect in Virginia, and December, 1989, blood from 1025 suspected drug-impaired drivers was analyzed. Cannabinoids (211 cases), phencyclidine (200) and cocaine/benzoylecgonine (148) were found with greatest frequency. There was no detectable alcohol in 70% of the PCP users, 48% of the cocaine users and 44% of the marijuana users. The average age of drivers in whom PCP, marijuana or cocaine were found was 26 (range 16-52) and 87% were male. In 23% of the DUID cases, the blood alcohol was 0.10% or greater. Drugs were found in 65% of the cases that had blood alcohol levels less than 0.10%. In 14% of the DUID cases, neither drugs nor alcohol were found. In most cases, only a single drug or alcohol was reported. Less than 12% of the cases had 2 or more drugs other than alcohol reported. The participation in DUID enforcement was widespread, with cases submitted by 93 different agencies in 106 jurisdictions in 1989; however, 50% of the cases in 1989 were submitted by only four agencies. Drug Influence Evaluations conducted by Drug Recognition Technicians (DRTs) accompanied 119 cases in 1988 and 74 cases in 1989.

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DRUG CONCENTRATIONS AND DRUG IMPAIRMENT OF TRUCK DRIVERS.
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The National Transportation Board has reported that 57(31%) of 182 heavy truck crashes were probably caused by driver fatigue followed by alcohol and other drug use impairment of 53(31%) drivers.

Forensic experts would agree that 19(10%) drivers whose bloods contained 0.10% of alcohol were impaired.

It was reported that 17(9%) of the drivers were impaired by the use of one of the following: marijuana, cocaine, or amphetamines and 8(4%) by the use of combinations of these drugs. In my opinion, the mere presence of these drugs or their metabolites in blood or urine does not establish impairment by drug use with any scientific certainty. Cocaine and benzoylecgonine were found in 4 drivers and 9 other specimens contained benzoylecgonine.

The use of codeine, ephedrine, chlorpheniramine, pseudoephedrine, or phenylpropanolamine are cited as probable cause for impairment.

Drug concentrations found in the bloods of the above " drug impaired" drivers are similar to those reported in a study of live apparently unimpaired truck drivers.

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CURRENT ISSUES IN NIDA AND CAP FORENSIC PERFORMANCE TESTING CHALLENGES

Michael Lehrer, PhD, Chief of Biochemistry and Toxicology (Long Island Jewish Medical Center, New Hyde Park, NY 10042)

Workplace drug testing continues to generate controversy. One area of agreement between drug testing advocates and those opposing it is the need to avoid any possibility of false positive results. Governmental agencies and professional organizations have assumed leadership roles in helping laboratories meet the challenge.

The National Institute on Drug Abuse (NIDA) has initiated a laboratory certification program with a regulatory perspective. Unfortunately, NIDA has not released information regarding the performance of labs in their performance testing challenges. Other groups, including the College of American Pathologists (CAP), NY State and Pennsylvania, have released periodic reports summarizing proficiency testing performance. This feedback can be extremely helpful to labs in gauging their performance and that of the testing industry as a whole. Data from the CAP Program is especially helpful because of the large number of participating labs.

Examination of the data indicates that the rate of false positive and false negative results continues to be high in CAP surveys. GC/MS quantitative results reveal surprising trends. Proficiency data will be presented and its implications for drug testing labs will be discussed.

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THE POSTMORTEM REDISTRIBUTION OF TRICYCLIC ANTIDEPRESSANT DRUGS: A MECHANISM AND INTERPRETATION OF POSTMORTEM CASES

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Amitriptyline was determined to leach from several tissues postmortem with liver the major contributor to increasing blood concentrations in an in vivo rabbit model.

In vitro loss of amitriptyline from rabbit liver slices was not affected by temperature or pH in therapeutically dosed rabbits but loss was greater at pH 5.9 than either 7.4 or 8.0 in overdosed rabbits. Loss of amitriptyline correlated well with the loss of lactate dehydrogenase and ultrastructural changes in the cell morphology.

Tissue: tissue ratios and total tissue concentrations with establishment of cutoff values for therapeutic and overdose ingestions facilitated diagnosis of the involvement of tricyclic antidepressant drugs in human deaths.

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FACTORS IN THE POSTMORTEM INTERPRETATION OF COCAINE AND METABOLITE BLOOD CONCENTRATIONS

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The metabolism and hydrolysis of cocaine (COC) was studied in vitro, in vivo and postmortem to determine if concentrations of the COC metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME) would facilitate interpretation of COC related Medical Examiner cases.

COC hydrolyzed to EME, in vitro, in unpreserved whole blood. However, in blood preserved by a pseudocholinesterase inhibitor and in phosphate buffers, COC hydrolyzed to BE. COC was stable in blood adjusted to pH 5 and preserved with sodium fluoride and organophosphates for at least 150 days.

An in vivo study over time in subjects given two doses of either intravenous (IV) or smoked (SM) COC confirmed that BE is the principal metabolite of COC in blood. EME, when present, did not exceed 5% of the BE concentration. It appears that EME may arise in postmortem blood largely as a result of nonmetabolic hydrolysis of COC. Thus the EME concentration in unpreserved blood specimens could be added to the COC concentration to obtain an estimate of the actual blood COC concentration prior to in vitro hydrolysis, while BE could be attributable to normal COC metabolism.