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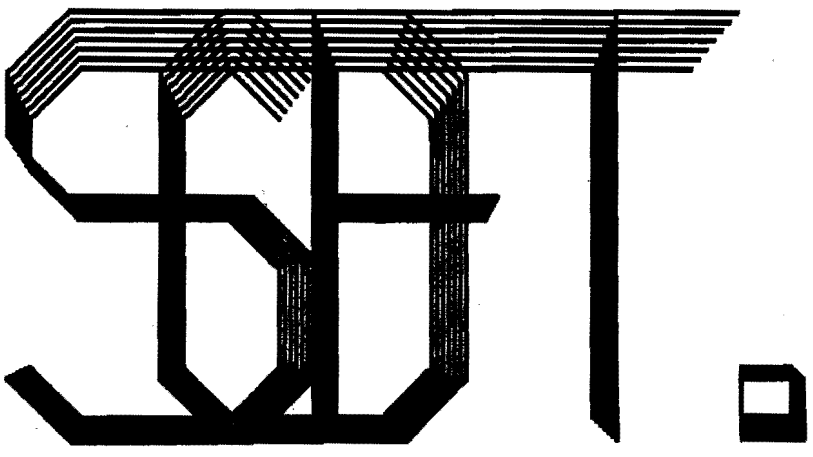

ABSTRACTS

Analysis of Morphine and Other Opiates by GC/MS/SIM.
JOSEPH J. SAADY, M.S., and ROBERT V. BLANKE, Ph.D.
MCV Hospital Toxicology Laboratory, Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Blood, plasma, tissue homogenate or hydrolyzed urine or bile is adjusted to pH 8.9. N-allylnormorphine (NAL) is added as an internal standard and the specimen is extracted with ethyl acetate:2-propanol (90:10). The organic solvent is evaporated and the opiates derivatized with N-methyl-bis (trifluoroacetamide) to form the ditrifluoroacetyl (TFA) derivatives of morphine (MOR) and NAL. The derivative solution is injected directly on to a 0.9 m x 2 mm glass column packed with 3% OV-101 and interfaced to a HP5985 MS/DS. The MOR di-TFA (m.w.=477) and NAL-di-TFA (m.w.=503) are monitored at m/z 477 and 503 respectively using electron impact. This procedure has proven to be both rapid and highly specific and can be applied to other, similar compounds.

Analytical Toxicology: Applications of Gas Chromatography-Dual Detection Systems for the Qualitative Analysis of Drugs in Biological Specimens.

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Dual detection systems consisting of flame ionization, nitrogen phosphorous, electron capture, and electrolytic conductivity detectors (FID, NPD, ECD, EICD) are used to obtain improved sensitivity and selectivity. Screening of a biological extract + C-10 to C-30 n-alkane internal or external standards is done by GC-NPD/FID; initial decisions are based on observed retention indicies of unknown peaks and nitrogen/carbon response ratios. When qualitative confirmation is done by GC, heteroatom/carbon response ratios are determined by isotherman GC vs. authentic standards. Chemical derivatization-GC-NPD/FID techniques may be used to confirm expected shifts in GC retention indicies and NPD/FID response ratios for some classes of drug metabolites. Qualitative and quantitative experiments to determine confidence internals, empirical prediction, and GC-detector quality assurance are discussed. Forensic analysis using multiple detector systems are illustrated.

Determination of Pentazocine and Tripelannamine (T's and Blues) in Postmortem Blood by Gas Liquid Chromatography with use of a Nitrogen Phosphorus Detector.

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We present a method for the quantitative determination of pentazocine and tripelennamine in blood samples obtained from T's and Blues addicts. The drugs were initially extracted from blood at pH 11 with benzene/isopropanol (9:1) solvent containing 0.1 mg/l of mepivicaine internal standard (I.S.), followed by extraction into 0.5N HCl. The drugs were then back-extracted at pH 11 into benzene/isopropanol solvent which was evaporated to dryness. The residue was dissolved in 0.050 ml of methanol and subjected to gas liquid chromatographic (GLC) analysis. All GLC determinations were performed on Perkin Elmer Sigma 2 gas chromatograph equipped with a nitrogen phosphorus detector (NPD). The detector response was recorded and integrated with a Perkin Elmer Sigma 10 Data Station. The drugs were chromatographed on a 1.8 m x 4 mm id glass column packed with 3% OV-17 on Chromasorb WHP, 80/100 mesh. Operating conditions were: temperatures; injection port, 250 C; column, 220 C; and detector, 275 C. Gas flows and/or pressures were: nitrogen carrier gas, 30 ml/min., 100 psig; air, 26 psig; and hydrogen, 12 psig, respectively. The concentration of pentazocine and tripelennamine in samples was determined by the method of internal standard utilizing electronic intragation of peak area. The retention time in minutes were: tripelennamine, 2.7; mepivicaine, 3.9; and pentazocine, 6.9. Standard curves were linear over a concentration range of 0.1 - 1.0 mg/l tripelennamine ($r=0.998$) and 0.5 - 5.0 mg/l pentazocine. ($r=0.999$). The within-run CV of extracted blood containing 0.2 mg/l tripelennamine and 1.0 mg/l pentazocine were 5.6% and 5.2%, respectively ($n=20$). The mean recoveries over the range of the standards were: tripelennamine, 101% + 3.6% ($n=12$) and pentazocine, 79.5% + 1.5% ($n=12$). Blood concentrations of the drugs found in T's and Blues addicts vary widely: tripelennamine, 0.03 - 1.0 mg/l and pentazocine, 0.04 - 3.6 mg/l.

Ketamine Found - Drug of Abuse or Accident??

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Ketamine is a "dissociative" anesthetic agent, structurally related to phencyclidine, that may be administered, intravenously or intramuscularly. The drug has the potential for street abuse due to its PCP-like hallucinogenic side effects. There are no reports of Ketamine deaths in the literature. Ketamine was unknowingly administered in hospital to a teen-age stabbing victim. The drug was detected during routine thin layer screening and subsequently identified. The concentration of Ketamine and its metabolite, norketamine, were determined in tissues by gas-liquid chromatography after heptafluorobutyric-anhydride derivatization. Derivatization was necessary to separate the parent drug from its metabolite. No Ketamine was found in the hospital admission blood. Was Ketamine the cause of death or did the mistake occur after the victim's heart had stopped beating?

A Chloroform Fatality

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This is a report of two chloroform fatalities. A Gas Chromatographic head space procedure using n-propylalcohol as an internal standard was used to quantitate the chloroform concentrations. Tissue concentrations, distribution and the analytical method of analysis will be presented and discussed.

Ethchlorvynol Metabolites or ?

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The TLC analysis of urine samples from clinical and forensic cases involving overdoses of ethchlorvynol resulted in the detection of large quantities of substances believed to be ethchlorvynol metabolites. The detection of these same urinary substances following excessive doses of chloral hydrate or vitamins suggested that the drugs, per se, were not the sources of these compounds. The identification of the urinary substances by chromatographic and spectroscopic methods will be discussed.

Urine Drug Extraction with a C_{18} Bonded Adsorption Column.

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A simple and rapid urine extraction has been developed for screening of acid, neutral, and basic drugs. Adsorption onto a non-polar C_{18} bonded phase column, elimination of chromogenic material, and subsequent drug elution with a single solvent is accomplished in minutes. The residue from the extracted sample can be split-spotted for thin layer separation of acid-neutral and basic-neutral drugs using acetone:chloroform (1:9) and ethyl acetate, methanol, and ammonium hydroxide (170:20:5) solvent systems, respectively. Confirmation of the thin layer positives is afforded by GC/MS on either the unused extract or new extract. This procedure has been utilized for routine analyses of urines in drug screening. It was found to be more efficient than other adsorption and resin columns yet faster by two-thirds than comparably efficient liquid-liquid extraction procedures.

Lidocaine Toxicity.

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Lidocaine is one of the most prescribed drugs, especially in emergency resuscitative situations. It is known to be a highly effective and useful antiarrhythmic. The potential for overuse and resulting side effects is always present with any popular successful drug. Therefore, its course must be continually examined. This research has studied human brain and blood levels for over one hundred Medical Examiner cases, followed by a parallel animal model. The animal model demonstrated respiratory arrest at doses less than those required for cardiac arrest. Fluorescent antibody studies showed lidocaine localization in medulla-pontine neurons indicating this site for possible lidocaine toxicity. It is these aspects of the drug which are covered in this research study.

Rapid HPLC Method for the Quantitation of Haloperidol (Haldol) in Human Plasma.

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Haloperidol (Haldol) is an antipsychotic major tranquilizing agent. It is administered in situations of acute crisis and chronically for the treatment of schizophrenia. Haldol and the internal standard chlorohaldol are extracted from 2 ml of alkalized plasma (pH=9.5) with 5 ml of 95:5 hexane: ethylacetate. The organic phase is mixed with 0.3 ml of 0.4% (v/v) glacial acetic acid and 0.1 ml of the aqueous phase is injected onto the HPLC. Analysis is carried out on a C_{18} reverse phase column under isocratic conditions (50:50 acetonitrile: 0.01 M sodium acetate, pH=4) and wavelength monitored at 254 nm. The coefficient of variation for the method is 3.0% and is sensitive to 5 ng/ml of plasma. For patients on a chronic dose regimen, plasma levels of Haldol are typically 20 ± 15 ng/ml.

The Analysis of Fresh and Fixed Tissue Specimens For the Presence of Quarternary Amine Neuromuscular Blocking Agents.

JESSE H. BIDANSET, Ph.D., W. CHRISTOPHER LONG, Ph.D., WILLIAM C. BRESS, M.S., JOSEPH BALKON, Ph.D., VINCENT D. LYNCH, Ph.D.
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Fatal doses of curare, pancuronium and gallamine were administered by subcutaneous injection to rabbits. The animals were subsequently autopsied and samples of blood, brain, liver and kidney were obtained. Portions of these tissues were immediately analyzed in order to establish a tissue distribution. Fixed samples (stored in formaldehyde for 6 months) were similarly extracted and analyzed. A paired-ion extraction was used for isolation. The extracts were then analyzed by reverse phase HPLC using a C-18 ODC silex column. Various developing solvents for HPLC were examined. Eluates, separated by HPLC, were collected, freeze-dried and submitted to direct probe mass spectrometry.

High Performance Liquid Chromatographic Method for Quantitating Barbiturates.

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Numerous HPLC methods have been published for the quantitation of anticonvulsant drugs and barbiturates. However, most of these methods employ buffered mobile phases which require lengthy column flushing procedures to prevent blockage. A HPLC method for barbiturates is presented here using methanol: water (52:48) as the mobile phase. To 0.5 ml of blood, serum, or plasma is added .05 ml of internal standard (200 mg/l barbital) and 0.5 ml of phosphate buffer (pH=5.5). This is then extracted with 5 ml of dichloromethane and mechanically rotated for 5 minutes. The organic layer is removed and filtered into a conical tube and evaporated to dryness. The residue is reconstituted with 0.2 ml of mobile phase and 20 ml was injected into the HPLC. A Hewlett-Packard 1080 liquid chromatograph was used. The column was a Chromanetics C18 column (10μ particle size) maintained at 35 C. The flow rate was 1.2 ml/min and the variable wavelength detector was set at 220 nm. Linear standard curves were established up to 60 mg/l for amobarbital, butabarbital, pentobarbital, and secobarbital and up to 120 mg/l for phenobarbital. This method could detect quantities as low at 0.2 mg/l. Retention times of other co-extracted drugs are provided. Within day and day to day precision were determined.

Acute Methanol Intoxication.

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A 35 years old male was transferred from another area hospital where he had walked in in a drowsy and unsteady state complaining from a day long headache, abdominal pain, nausea and vomiting. Later he went into respiratory depression, coma and severe metabolic acidosis, pH 6.9. Blood toxicology revealed evidence of Methanol of a 542 mg/dl level. He was given 12 ampules (about 50 gms) sodium bicarbonate then transferred to our VAMC. On admission, the patient was deeply comatose and unresponsive, with a non-reactive very small fixed pupils. Methanol was 572 mg/dl and blood gases showed pH 7.18, PO_2 376 mm Hg, Saturation 99.3% PCO_2 27 mm Hg, HCO_2 9.6 meq/L, serum osmolality

512, CO_2 12 meq/L. Other lab tests were within normal range. Patient was treated with Hemodialysis (HD) and ethanol IV, put on MA1 respirator, and a frequent electrolytes and ABG's monitoring. In spite of HD (3 times), ethanol, bicarbonate and other supportive therapy, the patient remained deeply comatose and unresponsive. His last documented Methanol and ethanol levels were 36 and 58 mg/dl respectively. He had been arrested nine times and resuscitated successfully except for the last one. Patient died 36 hours after his admission.

Perspectives in Industrial Toxicology.

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It is the concern of industrial toxicology to assess the potential for chemicals to produce adverse effects to human health in the following situations: (1) during normal operations and under conditions of overexposure in the workplace, (2) as a consequence of recommended use and possible misuse by consumers, and (3) when discharged into the general environment. This review will cover the pressures and needs for toxicological and related studies; health-based regulations covering the chemical industry; routine and specialized toxicological test procedures; definition of exposure guidelines and the need for protection of workers; methods for communicating occupational health and product safety information; and current problem areas in industrial toxicology.

Qualitative Analytical Screens for a Broad Spectrum of Pesticides in Human Fluids and Tissues.

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"Pesticides", as defined by the EPA is a very broad term including insecticides, rodenticides, herbicides, fungicides and other chemical agents used to control or kill whatever life form man deems to be a pest. For the purposes of this paper, which is to examine approaches to pesticides screening, the broad categories are chlorinated hydrocarbons, organophosphates and carbamates. Other chemical groups of pesticides will be treated as individual examples. The objective of pesticides screening in the analytical

toxicology setting are: (1) to uncover significant occupational exposures, (2) to detect inadvertent exposure, (3) to confirm histories of deliberate or accidental exposure, (4) to cover the necessary ground in "general unknown" testing. Chlorinated organic hydrocarbons, of which DDT is the most familiar (although not presently distributed in the United States), are screened most appropriately by gas chromatography with electron capture detection. The gas chromatographic behavior of DDE, chlordane, aldrin, dieldrin, kelthane, endrin, methoxychlor, toxaphene and other chlorinated pesticides has been examined with the OV-1 GC phase. A method for extraction, clean-up and gas chromatography of DDE-type pesticides will be presented. Organophosphates and carbamate pesticides are appropriately screened by thin layer chromatography. Quantities of down to 0.1 mcg/ml urine can be detected with selective reagents such as 4(p-nitrobenzyl)pyridine and 2,6-dibromo-N-chloro-p-benzoquinoneimine, which have specificity towards organophosphates and carbamates, respectively. Specific screening tests are necessary for paraquat and diquat. These substances are separated from urine or blood by cation exchange chromatography and detected and quantified colorimetrically. Indandiones, such as diphacinone, may be screened by visible-ultraviolet spectrophotometry, as they are strongly absorbing molecules.

Butyl Nitrites - A New Area of Concern for Public Safety.

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The constraints placed upon the availability of amylnitrite (by its return to prescription status in 1969) have led to the widespread abuse of butyl nitrite(s). The most common, of the nitrous acid esters of the four butyl alcohols (iso-, n-, see-, tert-) is isobutyl nitrite; estimated gross sales in 1980 were projected to be $40 million in the United States. Virtually no animal or human toxicity data for any of these compounds were available as recently as 1978. Comparative studies of i.p. LD50 values in mice showed a delayed toxicity for the t-butyl

compound and ranged from 158 to 613 mg/kg. When administered by gastric intubation, the LD50 values ranged from 171 to 428 mg/kg in mice, and when determined by the inhalation route in mice, the median LD50 values ranged from 567 to 10,852 ppm. In subchronic exposure studies (7 hr/day x 60 days), at levels that resulted in >85% survival, all four butyl nitrites produced significant weight gain impairments over the first 30 days; only with t-butyl nitrite was this impairment continued for days 31-60. Histopathological examinations revealed increased organ weights and some alterations in liver function. Methemoglobin saturation levels at the end of selected daily exposures to isobutyl nitrite were consistently in excess of 10 times control values after day 1 of the study. The data support the conclusions that the butyl nitrites have a significant toxicity; their use as recreational drugs is not without hazard. (This research was supported in part by NIH Grant GM 07095.)

Aspects of Forensic Occupational Toxicology.

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Litigation and administrative-law hearings related to alleged harmful occupational exposure to dangerous chemicals and toxic substances are now frequent occurrences, and are a legitimate outlet for forensic toxicology contributions. Some striking differences exist between these legal actions and the typical postmortem medical examiner's toxicology case: 1) Most commonly, the alleged injury involves a living subject and the extent, permanence and consequences of the resultant health damage or disability from the exposure is a principal issue; 2) civil discovery procedures govern and are often employed in attempts to commit the expert to stated findings and conclusions prior to the trial or hearing; 3) usually, several federal and state regulatory agencies have concurrent jurisdiction over the workplace, activities, and substances involved - and discovering the applicable regulations and the extent of compliance with them at the time in issue is a formidable challenge; 4) there is often a long time

interval between the allegedly harmful events and the resultant litigation, with no present opportunity to establish by chemical analysis the presence or concentration of the chemical entities involved; 5) the typical case was not initially considered from a forensic point of view, and the investigative procedures and records of the physicians and hospitals involved reflect that absent focus; 6) the role of the toxicological expert most often involves interpretation and evaluation of the findings and conclusions *of others rather than the expert's own* toxicological analysis results. In the light of these factors, suggested practices in this subspecialty will be offered for the practicing forensic toxicologist, together with suggested information sources and proposed questions and issues for case elucidation.

Toxicology of PCBs.
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Polychlorinated biphenyls (PCBs) constitute an important group of synthetic chlorinated compounds with considerable industrial utility and surprisingly little human toxicity data. Electron capture GC procedures for PCBs at the PPB concentration level permit determination of control and exposured population exposure to various commercial Aroclors. This paper discusses both controlled and uncontrolled studies involving chemical hazardous waste, heavy maintenance and unexposed workers involved with PCBs. These studies show an inconsistent relationship of plasma PCB levels to findings of clinical toxicity. The data do show a consistent elevation of fat PCB levels as a more accurate measure of cumulative PCB exposure.

Severe Neuropathy from Pesticide Spray.
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This is a case report of a 25-year-old Black male who was exposed to airborne pesticide spraying one and half hours per day for five (5) consecutive days. Four (4) days afterwards the patient developed nausea,

vomiting, and abdominal pain. Ten (10) days afterwards he developed numbness and paresthesias of his distal extremities. Evaluation at Cook County Hospital three (3) weeks later revealed hyperkeratosis of the palms of both hands and a severe motor and sensory peripheral neuropathy on physical examination. A Hair Arsenic level of 1400 micrograms per gram of hair, a Urinary Arsenic level of 6.2 milligrams per liter and a Blood Arsenic level of 20.9 micrograms per decaliter was obtained. Subsequent information revealed that the pesticide contained Nitroseb (a dinitrophenol), M.S.M.A. (Monosodium methylarsonate) and a surfactant.

The Matter is Thallotoxicosis.

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In December, 1971 a 77 year old female was admitted to a small hospital in downstate Illinois complaining of gastric pain. The diagnostic work-up revealed a myocardial infarction. Recovery was normal until suddenly 16 days after admission she again had severe gastric pain and vomiting, which led rapidly to coma and death. Autopsy revealed: 1) generalized arteriosclerosis, severe arteriosclerosis in the coronaries, aorta and cerebral vessles, 2) generalized hemorrhagic duodenitis without ulceration. The death certificate was filed indicating a natural death and the body interred in the local cemetary. One year later it was noted by the sheriff's police that other members of the family were also ill, complaining of gastric pain. A heavy metal screen on these patients revealed the presence of high levels of thallium. In June 1977 after a long investigation including several deaths in the family, an exhumation order was obtained on the body and a second autopsy was performed 6 years after the original. A description of the second autopsy, thallium levels and methodologies are presented.

Experiences in Blood Lead Analysis.

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The Medical Laboratory of Drs. Thornton, Haymond, Costin, Buehl, Bolinger, Warner, McGovern, & McClure, Indianapolis, Indiana

Blood lead analyses have been part of the routine workload in our laboratory for the past six years, and we wish to share some of these experiences. Areas to be discussed will include 1, Methodology--Past and Present; 11, Quality Control; and 111, Proficiency Testing.

Determination of Trace Elements in Biological Tissues By Energy Dispersive X-Ray Fluorescence.

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A micro energy dispersive x-ray fluorescence (EDXRF) system has been employed for the simultaneous analysis of trace elements in various animal tissues including serum, urine, hair, muscle, liver, brain, kidney, and prostate. The technique is based on the detection of photon energy produced by the collision of high energy monochromatic x-rays with inner orbital elections of elements being analyzed. Work is presently being conducted to establish the distribution of S, ca, Ti, V, Cr, Mn, Cu, Fe, Zn, Hg, Pb, As, Se, Br, Ni, Rb, Sr, and Mo in normal human tissues as a prerequisite to the study of possible changes due to disease. A preliminary study comparing the concentrations of trace elements in mormal prostate with prostatic tumors indicate significant elevations of Fe, Cu, Se, Br, and Cr. There are marked decreases of Zn and Mn in prostatis tumors. Conversely, manganese in prostatic tissue increased three fold following castration. This effect was reversed by hormonal therapy. Data will be presented showing the various distributions of trace elements found in serum, urine, hair and various organs.

Thin-Layer Mass Screening Detection of 11-Nor-delta-9-Tetrahydrocannabinol-9-Carboxylic Acid in Human Urine.

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A simple, efficient, specific, reproducible and cost effective Thin-layer Chromatographic procedure for the detection of marijuana use in the urine of marijuana smokers has been developed. The test involves total extraction of free and conjugated 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid(THCA) at a pH of 3 to 4 after a very mild alkaline hydrolysis of the urine specimen. Thin-layer chromatographic separation is achieved on a 20 x 20 cm Gelman precoated silica gel glass micro-fiber sheet. The sensitivity of this proposed procedure is 50-100 ng/ml of urine and volume of urine needed is 20 ml. The efficacy and the sensitivity of the procedure were tested on the urine specimens collected in controlled study by smoking street marijuana. The proficiency of the test was evaluated by applying the procedure to the urine specimens shipped by the Center for Disease Control, Atlanta in its 4th survey of 1980 and the 1st survey of 1981 with 100% accuracy. The specimens were spiked with 100 ng and 150 ng of THCA per ml of urine. This laboratory analyzed more than 100 urine specimens using enzyme multipled immunoassay technique (EMIT) and all the positives obtained by the EMIT system were analyzed using the proposed TLC procedure. The results were 100% in agreement for the positives shown by the EMIT system. The procedure can detect the presence of marijuana in the urine of smokers who either smoke a few puffs of street marijuana though a tobacco pipe or smoke 1/4th of a marijuana cigarette (equivalent to 200 mg of street marijuana) for recreational purposes.

Monitoring Delta-9 Tetrahydrocannabinol Concentrations in the Blood of the Impaired Driver.

DWIGHT A. HORNBACHER
Bio Lab Medical Group, Colton, California

A triturated RIA procedure was used to determine delta 9 tetrahydrocannabinol concentrations in forensic blood samples. Samples obtained within 2 to 4 hours after initial intake did reflect a correlation between

level and presenting symptoms. After 8 hours there appeared to be no direct relationship between symptoms and delta-9 THC levels in blood. Possible reasons for the phenomenon will be discussed.

Determination and Significance of Cannabinoids in Blood and Urine.

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Methods for the determination of cannabinoids in biological specimens, particularly blood and urine, will be evaluated. At the present time, the immunoassays appear to offer the most to the forensic scientist. They do not require lengthy sample preparation that may result in losses, equipment is usually available, and they have adequate sensitivity and specificity. The disadvantage is that the tests are only presumptive and if absolute identification is necessary, an alternative confirmatory method must be used. The significance of qualitative and quantitative results obtained from plasma, blood, and urine will be discussed.

Metabolism of Δ^1-Tetrahydrocannabinol, A Review.

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Of the known marijuana constituents, only Δ^1-THC and Δ^6-THC are psychoactive. One marijuana cigarette will elevate blood Δ^1-THC levels to an average maximum of 25 ng/ml 15 minutes after smoking stopped. The low blood levels can partially be ascribed to the high lipid solubility of Δ^1-THC, 63% being bound to the lipoprotein fraction. Δ^1-THC is rapidly metabolized in the human body and appears to be eliminated in a biexponential fashion with a terminal half life of 56 h for non-users and 27 h for chronic users. Only 8% of the dose is excreted unchanged in the urine. The primary blood metabolite 7-hydroxy-Δ^1-THC is formed within minutes and appears to be as equally active as the parent compound. However, similarily to Δ^1-THC, excrements contain very small amounts of 7-OH-Δ^1-THC. The bulk of

excreted metabolites consist of polyhydroxylated metabolites and glucoronic acid conjugates. The majority of Δ^1-THC and metabolites are excreted in the urine by the rabbit and in the feces by the rat. Man appears to be intermediate between the two species in that 33% is excreted in the urine and 67% in the feces. A major urinary metabolite in humans is the Δ^1-THC-7-oic acid which is possibly produced via the α,β unsaturated aldehyde intermediate. Possible metabolic pathways and their long term implications will be discussed.