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TOXICOLOGY REPORT

A Review: 1999 to 2001

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ABSTRACT

Forensic Toxicology has remained a dynamic science since the early milestones in drug and poison detection more than 500 years ago. Paracelsus, in the sixteenth century, spoke of the "Toxicon", the toxic agent, as a chemical entity and was quoted as saying that "dosage alone determines poisoning" (1). This principle has been repeatedly affirmed over the intervening years as the relationships between the chemical structures of medicinals and their therapeutic activity and toxicity unfolded. Consequently, the modern Toxicologist has evolved into a true expert in the field, not only capable of analyzing body fluids and tissues and quantifying any drugs and poisons found, but as the expert in the interpretation of the effects of such chemicals on the human body, often establishing the cause of death or impairment. Change in all areas of science occurs at an ever accelerating pace, and Toxicology is no exception. The discipline of Forensic Toxicology grows in complexity with the appearance of new drugs, both on the pharmaceutical market as well as in the area of recreational use or abuse. Concurrent technical advances in methodology in terms of sensitivity, reproducibility and specificity have enabled the Toxicologist to keep pace. This review will focus on the advances of the last three years.

INTRODUCTION- CURRENT ISSUES OF CONCERN TO THE TOXICOLOGIST.

Discussions with contemporary Forensic Toxicologists about the current state of their science will generally surface several common themes.

New Drug of Abuse Patterns:

One must be cognizant of recently introduced chemical agents focussing on those which may be commonly used or abused. Are they considered potentially toxic, mood altering or impairing? Are current analytical methods capable of isolating and identifying such drugs or must new procedures be developed to cope with such chemicals? Of particular interest to Toxicologists are the changing patterns of drug abuse in general. Over the last several years the analysis for 'rave' drugs such as Ecstasy (MDMA - methylenedioxymethamphetamine) and the particular use of drugs such as benzodiazepines and gamma hydroxy butyrate (GHB) for sexual assaults have gained notoriety and special interest (2-14). Toxicologists must be aware of trends in drug abuse patterns such as the emergence of methamphetamine as a drug of choice for abuse with the concurrent problem of clandestine laboratory production. Recreational drug use patterns vary by geographical location. For example cocaine abuse had been largely a North American problem until recent years, but now is increasingly encountered in Europe and Australia as well.

New pharmaceutical drugs are approved for human use regularly and some of them are not readily detectable using routine methodology. As well, the illicit market produces new agents even as laws are passed to prohibit those currently in use. The term 'designer drugs' has been used to describe the clandestine production of illicit recreational drugs. Information on chemical syntheses is widely available on the Internet as well as in publications. Toxicologists must then do research, often on the fly, as they encounter new drugs in casework, in an attempt to identify the chemical agents and their metabolites, and to give some opinion as to the effects of these chemicals on the human body.

Method development/modification is continuous in toxicology. The resulting enhancement of sensitivity has progressed to the point that extremely low dosage drugs such as LSD are now detectable in small forensic blood samples (15,16).

Quality Control / Assurance :

Over the past years, quality in the workplace and in science has assumed a high priority. Organizations such as the American Society of Crime Laboratory Directors (ASCLD) and the International Standards Organization (ISO) through member agencies such as the Standards Council of Canada (SCC) have accepted the task of the accreditation of forensic Labs. Consequently, properly documented training of forensic specialists, proficiency testing of Labs and individual scientists as well as development and validation of methodology (17-22) are some of the areas which are coming to the attention of the Courts and client agencies. Laboratory safety is also a growing concern (23-26) for Toxicology and appropriate resources must be allocated to this issue.

Scientific Literature :

There has been a rapid growth in literature references pertaining to all aspects of forensic toxicology for more than the last decade. One key textbook, "Disposition of Toxic Drugs and Chemicals in Man"(27) summarizes pertinent information on each commonly encountered substance available (at the time of publishing), and forms a readily accessible reference source for interpretation of results. In terms of drug abuse, some excellent reference books and periodicals have appeared in the last few years (28-32). The use and abuse of alcohol continues to generate significant numbers of papers (33). One interesting area of research and application is the analysis of biochemical markers in blood for detecting alcoholism. This application is being used to detect problem drinking in suspected impaired drivers, especially repeat offenders. This information can be used to direct the driver into the appropriate treatment regimens.

Professional Liaison :

Toxicologists hold meetings of their professional organizations regularly. These meetings create a forum for discussion and exchange of ideas which are invaluable in assisting scientists to cope with new developments in the field. In Canada, the Canadian Society of Forensic Science (CSFS) has some 700 members, of which approximately140 are in the Toxicology section. In the present situation of limited financial resources, Toxicologists the world over find it difficult to attend meetings with any regularity. Communication using services such as the Internet will therefore become more prevalent. Organizations such as The International Council on Alcohol, Drugs and Traffic Safety (ICADTS), The International Association for Chemical Testing (IACT), The International Association of Forensic Toxicology (TIAFT), Society of Forensic Toxicologists (SOFT), American Academy of Forensic Science (AAFS), California Association of Toxicologists (CAT), the American Board of Forensic Toxicologists (ABFT), La Societe Francaise de Toxicologie Analytique and the CSFS all have Internet Web sites.

Stewardship of Resources :

In full forensic facilities, where Toxicology co-exists with other specialty areas such as explosive investigations and gunshot residue analysis, expensive instrumentation must be purchased. Cost effectiveness dictates that instrumentation should be shared by more than one forensic discipline if possible. One example is the shared use of mass spectrometry for drug identification in toxicology, as well as in explosive residue analysis.

In forensic toxicology, limited funding for resources, both human and analytical is the norm. Toxicologists must employ some form of risk assessment at each stage of the analysis to determine the probative value of every step in a sequential analytical operation. Likewise, diligent efforts must be made to obtain the complete picture from the police investigator and the pathologist in order to enable the analyst to focus on the drugs or chemicals most pertinent and plausible in the particular case.

Impairment by Alcohol:

Impaired driving by alcohol continues to be a major problem in developed countries of the world and is becoming a significant problem in developing countries. Some progress has been achieved in the latter part of the 20th century with the decline in incidence of impaired driving (34). That trend still continues, but authorities are monitoring the situation and continuing their efforts to lower the rates even further. They are especially concerned that the rates may rise again in the face of public perceptions that the problem has been largely solved. In Canada, Parliament recently undertook a review of the impaired driving laws (35). As a result, some changes in penalties were enacted and further studies into the problem of drug impaired driving were recommended.

Analytically, breath testing for alcohol has been the method of choice in North America and other countries for some considerable number of years (36-38). This method is growing in favour with European countries where blood testing had been the norm. With this growing international use of breath testing, international standards for the manufacture of evidential instruments were created through the Oganisation Internationale de Métrologie Légale (OIML) (39). This organization is currently working on standards for portable evidential instruments for use at roadside.

In the United States the use of passive alcohol sensors (PAS) is growing in popularity among police agencies (40). These battery-operated devices in the shape of flashlights detect alcohol in the ambient air surrounding a subject□s mouth. They incorporate electrochemical sensors (fuel cells), similar to the evidential instruments that incorporate fuel cells as the detector system for alcohol.

Impairment Due to Drugs:

The analysis of drugs in cases of suspected impaired driving has also come to the forefront through increased public awareness of this issue. While impairment by alcohol is still the dominant global problem, impairment by drugs is increasingly more common. The significant increase in the sensitivity of methods of drug detection allows the identification of even the lowest dosage drugs.

The Drug Recognition Expert (DRE) program is widespread in the USA and has been successfully introduced into Canada (41-44). The program educates the police officer in recognizing signs and symptoms of impairment by particular drug groups with subsequent presentation of such evidence in Court. While the Forensic Toxicology lab may not be involved in all these cases, confirmatory analysis is often necessary to substantiate the DRE□s opinion of drug impairment. Interpretive evidence concerning the effects of drugs on the ability to drive typically becomes the Toxicologist□s responsibility.

The Drug Recognition Expert program (DRE) was developed first in Los Angeles in the early 1980's, and has spread from California to at least 34 States and has been adopted in one Province in Canada (44). In the program, police officers undergo training to enable them to recognize drivers who are impaired by drugs and to determine the substances that are active in the driver. Extensive studies were conducted under the auspices of the National Highway Traffic Safety Administration (NHTSA) of the U.S. Department of Transportation. The end result was that officers so trained were correct in their identification of the impairing drug/drug group in most instances.

Canada has had legislation for many years making it illegal to operate a motor vehicle when one□s ability is impaired by a drug. However, many jurisdictions do not have legislation in place to deal with impairment by drugs, although some States in the U.S. and a few other jurisdictions now have lists of impairing drugs as well levels corresponding to impairment. In Canada at this time, the Toxicologist or other expert must make the association between the analytical results and the driving behaviour or pharmacological signs and symptoms in order to support an impaired by drugs prosecution.

Sexual Assaults Using Drugs:

Various central nervous system depressants, in particular, the short acting benzodiazepines such as flunitrazepam, triazolam and lorazepam as well as other agents such as GHB are being used to "drug" victims, especially in combination with alcohol (45). Historically, alcohol has been the most common agent used due to its prevalence in society and still remains the most frequently encountered pharmacological agent in drug facilitated sexual assault cases. However, the past several years in North America have seen a significant increase in the use of flunitrazepam (Rohypnol, 'Roofies') in cases of sexual assault. Typically, a victim will unknowingly ingest the drug in food or drink, then fall prey to sexual assault. The victim awakens sometime after the incident with little or no memory of the offence. Frequently it is the amnesia itself or unfamiliar surroundings that indicate to the victim that a sexual assault has occurred. The interpretive testimony of the forensic Toxicologist relating to the presence of the drug in the victim□s biofluids becomes of paramount importance in prosecution of the offence.

Workplace Drug Testing:

Various legislative requirements such as those of the transportation industry and of the military have given rise to workplace drug testing. Typically, cut off levels in urine are established for various drugs and metabolites. Members of the affected populations are subjected to random urinalysis, and the presence of a specific drug at concentrations above the respective cut off level will result in punitive actions. Cut offs are required to distinguish innocent exposure, poppy seed bagels, hemp shampoos etc, from deliberate drug use. Along with the growth in the urine testing industry, a secondary industry has developed. That activity is the development of commercial products deliberately designed to thwart drug testing. Oxidizing agents such as bleach, pyridinium chlorochromate and potassium nitrite have been used, and the use of adulterants in turn has spawned the growth of a tertiary industry to detect the presence of adulterants. Collaterally other techniques such as temperature and pH determinations and normalization to creatinine have been used to monitor the integrity of urine samples.

TOXICOLOGICAL ANALYSIS

The area of Toxicological analysis in the forensic field has been and continues to be extremely dynamic.

Initial Screen / Immunoassay:

It is normal for the toxicologist of 2001, when faced with a general unknown, to begin with screening techniques which are amenable to mass screening, and if positive, steer one toward individual target drugs which then must be extracted, confirmed and quantified.

Immunoassay techniques often form the first part of toxicological analysis. Not classified as extraction techniques, they usually precede extractions and the results may well dictate the extraction technique to be used. There are a variety of immunological screening methods available, including Radioimmunoassay (RIA), enzyme multiplied immunoassay technique (EMIT), enzyme linked Immiunosorbent assay (ELISA), cloned enzyme donor immunoassay (CEDIA), fluorescence polarization immunoassay (FPIA) including TDx, and ADx, and others. Recently an automated form of ELISA screening has been implemented and gained popularity, replacing older immunoassay techniques such as RIA and EMIT (46-53).

Immunoassay screening for certain drug groups, however, constitutes only a part of the total screening requirement. The remaining part of the screen sequence involving further extraction of drug groups is required and forms an ever increasing array of screening procedures for particular analytes.

Extraction Techniques :

The older methods of extracting drugs from body fluids and tissues, termed as liquid/liquid extractions, are still in common use (54,55) with recent innovations in robotic methodology being applied (56). Most classic liquid/liquid extractions depend on multi-stage treatment of the sample under carefully controlled pH conditions to facilitate the selective extraction of a drug or group of drugs. The scientific literature is replete with research reports of target analysis for single drugs or drug groups rather than broader all-encompassing approaches. Extractions are also done for specific drug or drug groups indicated by the results from the immunoassay screening method. These extracts are then subjected to other screening or testing such as gas chromatography (GC), high pressure liquid chromatography (HPLC), and more recently capillary electrophoresis (CZE) and mass spectrometry (57).

A more recent extraction process termed Solid Phase Extraction (SPE) has gained popularity (58-60), although it is most often directed at targeted or specific compounds. Robotic SPE systems, while expensive, provide advantage with the capability to process large numbers of samples quickly, accurately and safely. A further expansion of this technique involves collection of a sample on fibres or membranes (SPME) which leads to a cleaner extract for analysis (61-65).

Supercritical fluid extraction (SFE) and chromatography procedures continue to be developed and are finding increased usage in the forensic field in general (66-71). Another approach to extraction with great potential for analytical toxicology is the use of immunoaffinity extraction techniques (72) where target compounds are trapped by resin bound antibodies.

The preferred method of analysis for alcohol in body fluids continues to be head space gas chromatography (HSGC) (73). Sampling the head space above a prepared solution of body fluid eliminates the need to directly inject that solution onto a GC column with the resultant deposition of protein material on the column. Gas chromatography is preferred in forensic contexts since this procedure allows separation of alcohol (ethanol) from other common volatile substances (methanol, acetone, and isopropanol). Hospital labs routinely analyse blood samples for alcohol content in emergency room patients for diagnostic purposes using enzyme methodology (73).

This analysis is conducted on the serum portion which has a higher water content than the whole blood, and therefore a higher alcohol content than the corresponding whole blood. This relationship is unimportant in the hospital context, but becomes crucial when the patient happens to be a suspected impaired driver. Consequently, Canadian toxicologists find themselves increasingly asked to interpret for the courts these serum results since the *per se* law for alcohol (over 80) was written for whole blood, not serum. The use of SPME for alcohol and other common volatile substance analysis has been researched in recent years (74). So far, however, this method does not appear to have gained widespread favour in forensic toxicology.

Unconventional Samples :

Improved sensitivity of analytical techniques have made the detection, identification and quantification of drugs in unconventional matrices practical. Historically, urine, blood, vitreous humour and tissues were the only samples considered useful. Hair, nails, saliva, sebum, stratum corneum, sweat and other biological matrices have been determined to be useful samples for drug analysis, as either alternatives or complements to other samples (75-77). Urine and blood matrices provide relatively narrow time frames for drug use. A trend has been to use segmental hair analysis to expand the temporal window of drug use and to provide information on the chronology of drug use. A more recent trend is to monitor drug secretion in sweat by the use of patches applied to the torso or hands.

Instrumental Screening and Quantitative Techniques:

Following initial immunoassay and extraction procedures, extracts are subjected to various instrumental screening procedures designed to give some indication of specific drug presence. Preliminary identification of drugs within the extracts is normally done by chromatographic techniques. Quantification is done using GC, CZE or HPLC with incorporation of suitable quality control procedures. Mass spectrometry may also be used as a screening and quantification tool as well as a confirmatory procedure. Sensitivity limits have moved lower and lower with advancing technology. This applies to all modern techniques, from immunoassay procedures to GC, CZE, HPLC and the various hyphenated forms of Mass Spectrometry.

Where packed macrobore columns were in common use in both Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC) a decade ago, there has been a universal shift in GC to 15-30 m capillary columns and in, to a lessor extent, HPLC to microbore columns. These columns offer vastly increased sensitivity, improved separation and more accurate quantification. Drug detection using HPLC can employ various detectors, from photodiode array, fluorescence or electrochemical to thermospray MS, while GC detectors include flame ionization, electron capture, nitrogen phosphorus *(TSD)* and MS. The design of instruments changes quickly. Temperature and pressure programmable injection ports are now common in GC.

The advances in hyphenated techniques for mass spectrometry such as HPLC/MS (78-81) are quite promising especially with the appearance of tandem Mass Spectrometry. Capillary zone electrophoresis has a growing application in many areas of forensic analysis for Toxicology as well as potential in other disciplines such as Biology for DNA analysis (82-88).

Interpretation of Results and Future Trends:

Much has been published over the past decade concerning the effects, metabolism and pharmacokinetics of alcohol and drugs. Only a few examples of the multitude of developments in this area are discussed here. In general terms, the toxicologist attempts to answer questions such as: 'Are there any drugs, poisons or common volatile substances (usually referring to ethanol) present?', and 'what is the significance of that alcohol or drug concentration?'. Typically the toxicologist will answer the latter question by categorizing the concentration of a drug or drugs as therapeutic, toxic or lethal concentrations. For alcohol, the categorization extends to the specific concentration found, whether it be by breath or body fluid analysis.

The issue of impairment by drugs has grown vastly in significance over the years. The Society of Forensic Toxicologists along with the American Academy of Forensic Sciences have formed a joint committee known as the SOFT/AAFS Committee on Driving Under The Influence Of Drugs. Extensive drug monographs have been produced by this committee. The T2000 conference on alcohol, drugs and traffic safety held in Sweden in May 2000 had many significant papers presented on general and specific issues involved with the drug impaired driver. Papers included those focussing on specific drugs or drug groups such as cannabinoids, amphetamines, Ecstasy etc. (89-91), as well as legislative approaches to the problem from countries around the world. These include the 'zero tolerance' approach of Germany and Sweden (92,93) and the defined level legislation of Belgium (94). The countries of the European Union (EU) are participating in a study of the search for effective roadside testing for drugs through the ROSITA (Road Side Testing Assessment) project (95). The objective is to identify rapid, efficient, and reliable roadside tests that will facilitate improved police detection of drug impaired drivers.

In Canada, the Canadian Society of Forensic Science administers two committees under its Toxicology section which advise the Canadian Department of Justice on matters pertaining to legislation concerning impaired driving. The Alcohol Test Committee has been instrumental in the process of testing and validating breath testing devices, both screening and evidential units for use by Canadian law enforcement (96). Test results from the evidential units have been used in Canadian Courts for more than 45 years while roadside screening devices have been used for the last 25 years. The Committee continues to conduct research pertaining to new testing equipment prior to implementation under the Criminal Code of Canada. At this time, most Canadian Police jurisdictions are replacing their photoelectric devices with infrared instruments. The Drugs and Driving Committee was formed more recently and has been active in promoting legislation pertinent to drug impairment. It has also been active in introducing DRE programs in Canada.

Significant discoveries relating to the interpretation of drug concentrations found in post mortem samples due to the phenomenon of post-mortem drug redistribution are ongoing as is study of the effects of storage (97-98). Originally observed with drugs such as cardiac glycosides and the tricyclics antidepressants such as amitriptyline, the discovery of post-mortem changes in drug concentration revolutionized the field of post mortem drug interpretation. Simply put, drugs stored in muscle or body fat are released after death, elevating the surrounding blood drug concentrations significantly. Thus, if one discovers a lethal concentration of amitriptyline in post-mortem cardiac blood, a peripheral blood sample may indicate a lower, often therapeutic, level. Obviously in this case, the cardiac sample is misleading and interpretation must be based on the concentration in the peripheral sample. Work has been done in an attempt to learn where drugs concentrate and if a correlation can be made between concentrations of drugs in major organs and vessels. Results indicated that the highest concentrations were found in solid organs, and that databases based on concentrations from blood of unknown source are highly suspect.

Lastly, negative findings reported by the forensic toxicologist may spur re-examination of the autopsy report to determine if some natural disease condition capable of causing death has been overlooked. Some developments have come from actual case research in establishing cause of death which may be related to disease and/or natural causes. For example, the death of diabetics was previously difficult to prove as being related either to hyper or hypoglycemia. Now determination of glucose and lactic acid levels in the vitreous humour along with blood acetone determinations prove the existence of antemortem hyperglycemia in cases of sudden death.

Forensic toxicology will continue to play an important role in criminal investigations. The expanding use of drugs in sexual assaults will challenge the Toxicologist both analytically and in interpretation. Multiple drug use and abuse will add to the role of interpretation, especially in the area of drug interaction within the human body. The increasing pressure to de-criminalize the use of marijuana, especially for medicinal purposes, may potentially increase the amount of court time spent interpreting the effects of THC in operating a motor vehicle. All in all, forensic toxicology continues to be a field offering interesting and challenging tasks.

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