

Forensic toxicology concepts and applications in Pharmaceutical Medicine.

ABSTRACT

Forensic toxicology is the use of toxicology along with a few other disciplines like analytical, pharmacology, chemistry and clinical chemistry in medico-legal investigations of death, poisoning and drug use. The main aim of forensic toxicology is to understand the technology and the techniques that are used in deriving, analyzing and interpreting the results. This field of toxicology is not too concerned with the legal outcome of the investigation. The three main objectives of forensic toxicology are to establish the presence and identification of toxicant and ascertain whether they contribute to or caused harm or death. Substances that may affect a person's performance or behavior and ability to make rational judgement and substances that are not compliant with employment regulation or classified as substances of abuse. The field of forensic toxicology includes the measurement of alcohol, drugs and other toxic substances in the biological specimens along with the interpretation of the results in medico-legal contexts. A toxicological analysis can be done to various kinds of samples. The purpose of this review was to give an overview of the concept of forensic toxicology and its application in toxicity study and medicine. The different aspects of dead, living samples, and procedures for analysis has been elucidated, and the use of substances of abuse in forensic toxicology has been discussed

Keywords: Forensic toxicology, drug development, pharmaceutical medicine, post mortem, antemortem, doping substances

INTRODUCTION

Forensic toxicology is the use of toxicology and disciplines such as analytical chemistry, pharmacology and clinical chemistry to support medical or legal investigation of death, adverse drug reaction/poisoning, and drug use [1]. The primary concern in forensic toxicology is not the legal outcome of the toxicological investigation or the technology applied, but rather the outcome of the interpretation of results. A toxicological analysis can be used in generating different types of samples and interpretation of results [2]. A forensic toxicologist must consider the objective and scope of an investigation, especially any physical symptoms recorded, and any evidence collected at a crime scene that may orientate and narrow down the search, such as drug bottles, powders, trace residue, and any available chemicals [3]. Based on the information on the samples with which to work, the forensic toxicologist must determine which toxic substances are present, in what concentrations, pharmaceutical dosage form, and the probable effect of those chemicals on the victim [4]. There are many areas of specialty within the field of forensic toxicology the main field being the postmortem toxicology. The American Academy of Forensic Sciences (AAFS) emphasizes on the fact that the field of forensic toxicology is very collaborative in nature as a forensic toxicologist is often working in conjunction with law enforcement officers, forensic pathologists, forensic scientists, behavioural

toxicologists and other crime scene investigators [5-7]. The detection of the drugs and other toxic substances present in the biological samples are determined first by an initial screening and then a further confirmation of the identified compound and the quantification of the compound. Both the screening and the confirmation are done using different analytical methods validated by regulatory bodies based on norms and standards. [8].

To appreciate the importance of poison on human exposures the World Health Organization (WHO) has developed a rating chart for toxicity as illustrated in table 1. This chart is graded based on the amount of substance intake.

Table 1: WHO rating chart for toxicity [4]

Category	Toxicity	Concentration
1	Highly toxic and severely irritating	$\pm 1-5$ mg/kg
2	Moderately Toxic and moderately irritating	5-10 mg/kg
3	Slightly toxic and slightly irritating	50 -500 mg/kg
4	Practically non-toxic and non-irritant	± 500 mg/kg

In forensic toxicology investigation on an ideal homicidal and suicidal poison are studied and determined based on some characteristics as indicated in table 2.

Table 2: Characteristics of an ideal homicidal and suicidal poison [23, 45]

Characteristics	Suicidal	Homicidal
Accessibility	-	Cheap and easy
Antidote	Difficult	Difficult
Lethal dose	small	Small
Lethal period	small	Long
Taste	Pleasant	Pleasant
Signs/symptoms	Few or None	Resemble Disease
Examples	Cyanide, Opium, Insulin	Arsenic, Aconite, Barbiturates, Thallium, Organophosphorus, Madar, strychnine

1.1. Forensic Toxicology – Samples for identification.

There is the need for every analytical method in forensic toxicology to be carefully tested with a pre-forming validation of the method to ensure the correct and indisputable results each time. An accredited testing laboratory involved in forensic pathology has to respect regulatory requirements and policies that ensure the best possible results and safety of every individual [9]. The choice of method for investigation and testing mainly depends on what substance is suspected and the type of sample used for testing. In biological samples, many complex factors like the matrix effect, metabolism and conjugation of compounds have to be taken into consideration [3, 9]. Forensic toxicology attempts in finding out the compound statistics of the substances along with their concentrations.

Determining the substance ingested is often complicated by the body's natural processes within the context of absorption, distribution, metabolism and elimination (ADME), as it is rare for a xenobiotic to remain in its original form once in the body [10]. For example: heroin is almost immediately metabolized into secondary metabolites and further to morphine, making detailed investigation into factors such as injection marks and chemical purity necessary to confirm diagnosis [4, 11]. The substance may undergo some dilution by its dispersal through the body; while a drug or other regulated dose of a drug product may have grams or milligrams of the active constituent, and an individual sample under investigation may only contain micrograms or nanograms [12].

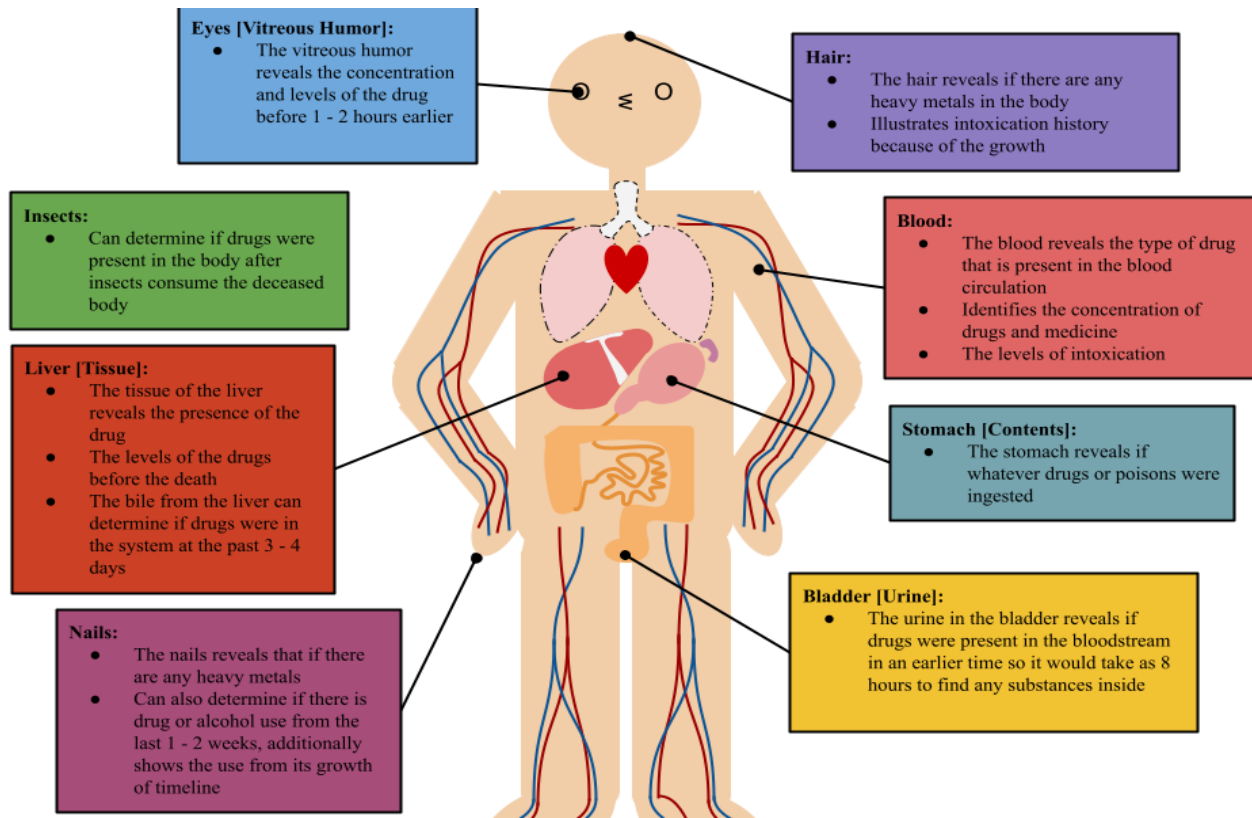


Figure 1. Drug's Location in the Body [9]

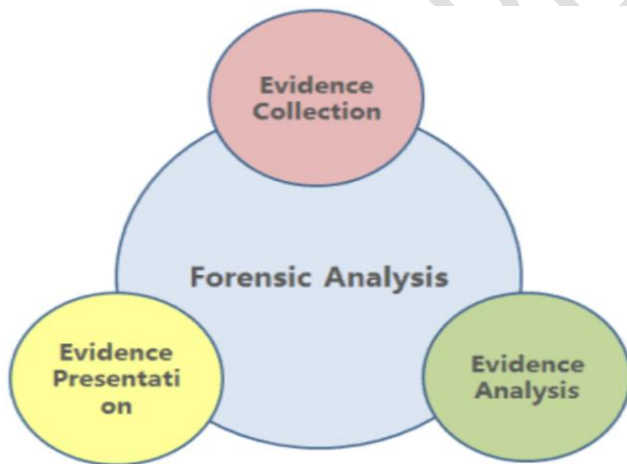


Fig 2. An illustration showing how forensic analysis is carried out [22]

1.1.1. Urine

A urine sample is urine resulting from the bladder and can be provided or taken post-mortem. Urine sample is very common among drug testing for employees and athlete [9]. A urine sample is quick and easy to obtain for live subjects. The use of urine samples provides the added advantage that they do not necessarily reflect the toxic substances the subject is under at the time of collection [10]. An example of this is delta-9-tetrahydrocannabinol (THC), is the main psychoactive substance found in the cannabis plant, from cannabinoid. For example, Marijuana use, which in heavy users can be detected in urine for up to 14 days following use. It can take as long as 8 hours until a given substance can be detected.

1.1.2. **Blood**

A blood sample of approximately 10 ml is usually sufficient to screen and confirm most common toxic substances. A blood sample provides the toxicologist with a profile of the substance that the subject was influenced by at the time of collection; for this reason, it is the sample of choice for measuring blood alcohol content in drunk driving cases [11]. A quantity of 10 cm³ of blood is required to screen and confirm the presence of most toxic substances. Unlike the urine samples, the blood sample screen provides the list of toxic substances present in the subject's body at the time of collection and is hence ideal for testing the blood alcohol content in drunken driving cases [3, 12].

A blood sample provides the toxicologist with a profile of the substance that the subject was influenced by at the time of collection, for the reason, it is the sample of choice for measuring blood alcohol content (BAC). BAC is most commonly used as a metric of Drunkenness for legal or medical purposes in drunk driving cases [13].

1.1.3 **Oral Fluid**

The layman term for oral fluid is saliva. However, oral fluid is a more appropriate term as saliva is only a component of the oral fluid. The concentration of the toxic substances present in the oral fluid is in parallel to that of the blood. The use of oral fluids is gaining prominence in the fields of clinical settings and drunken driving cases [11]. Oral fluid is the proper term, however saliva is used commonly. Saliva is a component of oral fluid. Oral fluid is composed of many things and concentrations of drugs typically parallel to those found in blood. Sometimes referred to as ultra-filtrate of blood, it is thought that drugs pass into oral fluid predominantly through a process known as passive diffusion [12]. Drugs and pharmaceuticals that are highly protein bound in blood will have a lower

concentration in oral fluid. The use of oral fluid is gaining importance in forensic toxicology for showing recent drug use, e.g. in clinical settings or investigation of driving under influence of substances [13].

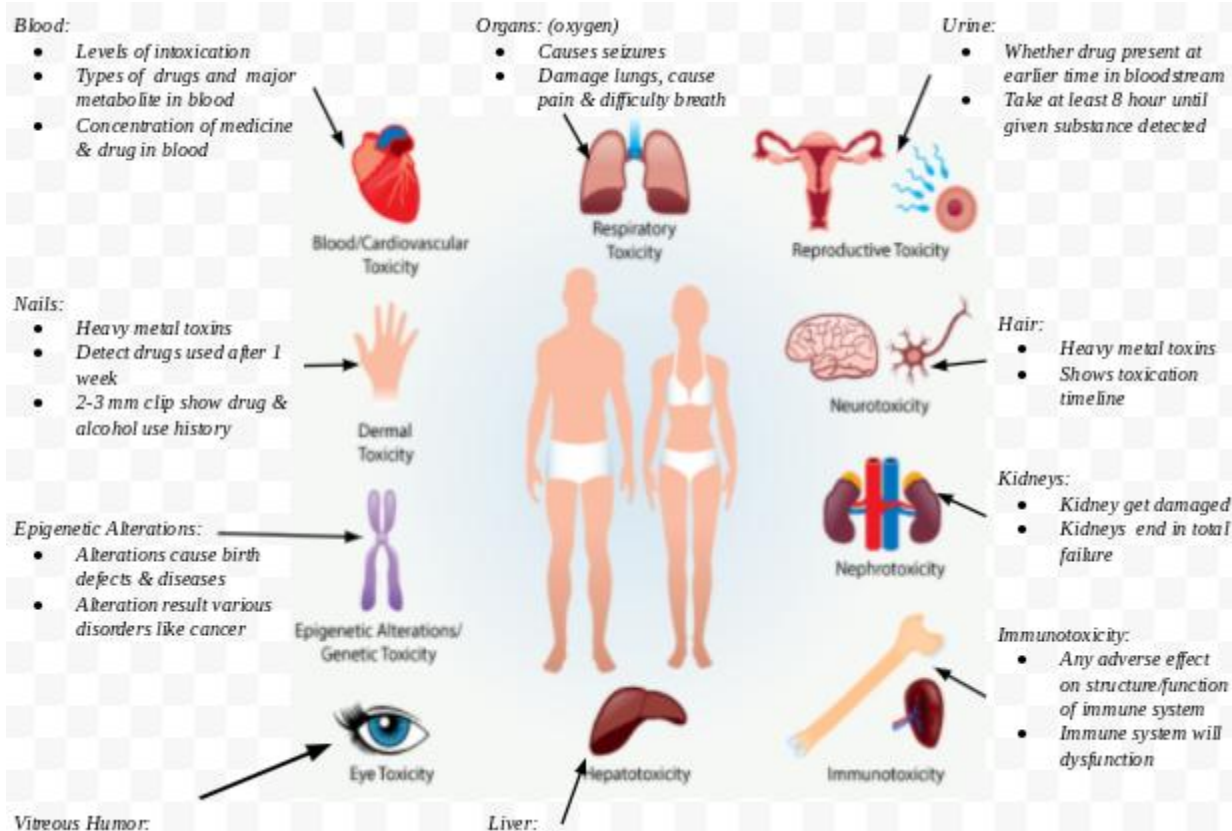


Figure 3. Drugs in the body [32]

1.1.3. Hair Sample

Hair is capable of recording medium to long-term or high dosage substance abuse. Chemicals in the bloodstream may be transferred to the growing hair and stored in the follicle, providing a rough timeline chronology of drug intake events. Head hair grows at rate of approximately 1 to 1.5 cm a month, and so cross sections from different sections of the follicle can give estimates as to when a substance was ingested. The hair is an important sample in

the field of forensic toxicology as it is capable of providing information about medium to long term history of drug abuse. This is because the chemicals in the bloodstream are transferred to the hair follicle via which a rough timeline regarding the intake of drugs can be deduced. As the hair grows at approximately 1.5 centimeters per month, the cross section of the hair at different intervals will give a rough estimate of when the drug was ingested.

Hair is capable of recording medium to long-term or high dosage substance abuse. Chemicals in the bloodstream may be transferred to the growing hair and stored in the follicle, providing a rough timeline of drug intake events. Head hair grows at rate of approximately 1 to 1.5 cm a month, and so cross sections from different sections of the follicle can give estimates as to when a substance was ingested. Testing for drugs in hair is not standard throughout the population. The darker and coarser the hair the more drug that will be found in the hair. If two people consumed the same number of drugs, the person with the darker and coarser hair will have more drug in their hair than the lighter haired person when tested. This raises issues of possible racial bias in substance tests with hair samples [1]. There are many other body fluids and body organs that are collected during an autopsy provide information that will be very vital to a forensic toxicologist. The common autopsy sample is the gastric contents of the subject that is very useful in the detection of undigested pills or liquids that were ingested just before the death. This sample is however not useful in highly decomposed bodies. Some of the other body parts that are used are the vitreous humor of the eye, brain, liver and the spleen.

A forensic toxicologist must consider the context of an investigation, in particular any physical symptoms recorded, and any evidence collected at a crime scene that may narrow the search, such as pill bottles, powders, trace residue, and any available chemicals. Provided with this information and samples with which to work, the forensic toxicologist must determine which toxic substances are present, in what concentrations, and the probable effect of those chemicals on the person. Determining the substance ingested is often complicated by the body's natural processes, as it is rare for a chemical to remain in its original form once in the body. For example, substance of abuse like heroin is almost easily metabolized into another substance and further to morphine, making detailed investigation into factors such as injection marks and chemical purity necessary to confirm diagnosis [17, 25]. The substance may also have been diluted by its dispersal through the body; while a pill or other regulated dose of a drug may have grams or milligrams of the active constituent, an individual sample under investigation may only contain microgram, or micrograms.

1.1.4. Oral fluid

The use of oral fluid is gaining importance in forensic toxicology for showing recent drug use, eg in clinical settings or investigation of driving under influence of substances. Oral fluid is the proper term; however, saliva is used commonly. Oral fluid is composed of many components and concentrations of drugs typically parallel to those found in blood. Sometimes referred to as ultra filtrate of blood., it is thought that drugs pass into oral fluid predominantly through a process known as passive diffusion. Drugs and pharmaceuticals that are highly protein bound in blood will have a lower concentration in oral fluid. [21]

Maggots and other organisms that may have ingested some of the subject matter may also ingested any toxic substance within it, referred to as entomotoxicology. Entomotoxicology is the analysis of toxins in arthropods mainly flies and beetles that feed on carrion. Using arthropods in a corpse or at a crime scene, investigators can determine whether toxins were present in a body at the time of death. This technique is a major advance in forensic, previously, such determination were impossible in the case of severely decomposed bodies devoid of intoxicated. Tissues or bodily fluid [27, 30]

Other bodily fluids and organs may provide samples, particularly samples collected during an autopsy. A common autopsy sample is the gastric contents of the deceased, which can be useful for detecting undigested pills or liquids that were ingested prior to death. In highly decomposed bodies, traditional samples may no longer be available. The vitreous humour from the eye may be used, as the fibrous layer of the eyeball and the eye socket of the skull protects the sample from trauma and adulteration. Other common organs used for toxicology are the brain, liver, and spleen. [33]. The inspection of the contents of the stomach must be part of every postmortem examination if possible because it may provide qualitative information concerning the nature of the last meal and the presence of abnormal constituents. Using it as a guide to the time of death, however, is theoretically unsound and presents many practical difficulties, although it may have limited applicability in some exceptional instances. Generally, using stomach contents as a guide to time of death involves an unacceptable degree of imprecision and is thus liable to mislead the investigator and the court. Characteristic cell types from food plants can be used to identify a victim's last meal; knowledge about which can be useful in determining the victim's whereabouts or actions prior to death [11, 24]

Some of these cell types include sclereids (pears), starch grains (potatoes and other tubers), raphide crystals (pineapple), druse crystals (citrus, beets, spinach), silica bodies (cereal grasses and bamboos) [33, 40].

In a case where a young woman had been stabbed to death, witnesses reported that she had eaten her last meal at a particular fast food restaurant. However, her stomach contents did not match the limited menu of the restaurant, leading investigators to conclude that she had eaten at some point after being seen in the restaurant. The investigation led to the apprehension of a man whom the victim knew, and with whom she had shared her actual final meal [13]. Time since death can be approximated by the state of digestion of the stomach contents. It normally takes at least a couple of hours for food to pass from the stomach to the small intestine; a meal still largely in the stomach implies death shortly after eating, while an empty or nearly-empty stomach suggests a longer time period between eating and death [4, 13]. However, there are numerous mitigating factors to take into account: the extent to which the food had been chewed, the amount of fat and protein present, physical activity undertaken by the victim prior to death, mood of the victim, physiological variation from person to person. All these factors affect the rate at which food passes through the digestive tract. Pathologists are generally hesitant to base a precise time of death on the evidence of stomach contents alone. What can be determined from vitreous humor in a postmortem analysis is that the vitreous can also be analyzed for glucose, ketones, alcohols, and certain drugs. A glucose level of <200 mg/dL is considered normal. Because glucose levels decline rapidly after death, a postmortem vitreous glucose level of >200 mg/dL is diagnostic of diabetes [24, 39]

1.1.5. The relationship between antemortem and postmortem drug concentrations in blood

The principles of postmortem toxicology, combine pharmacology and toxicology for the determination of how or if exogenous substances contribute to one's death. For this determination to be done postmortem and (whenever available) antemortem blood samples may be analyzed. Evaluating the relationship between postmortem and antemortem blood drug levels is important in order to better define an interpretive framework for postmortem toxicology [83, 85]. To do this, it is important to evaluate the differences in antemortem and postmortem drug concentrations, determine the role microbial activity and evaluate drug stability [44]. Microbial studies determined that the bacteria *Escherichia coli* and *Pseudomonas aeruginosa* could use the carbon structures of drugs as a source of food. This would suggest before sample collection; microbial activity could potentially affect drug levels. This process however would stop before toxicologic evaluation, as at autopsy blood samples are stored in tubes containing the antimicrobial agent sodium fluoride [86]. Analysis of preserved blood determined that under the current storage conditions sodium fluoride effectively inhibited microbial growth although in many instances inconsistent drug concentrations can be identified. When comparing antemortem to postmortem results, diphenhydramine, morphine, codeine and methadone, all can show significantly increased postmortem drug levels. In most cases, increased postmortem concentrations can be correlated with extended postmortem intervals. Other drugs, such as alprazolam, are likely to have concentration discrepancies when short antemortem to death intervals are coupled with extended postmortem intervals. While still others, such as midazolam can follow the expected pattern of metabolism and elimination, which in most cases often results in decreased postmortem concentrations [9, 87]. The importance of drug stability was displayed when reviewing the clonazepam/ 7-aminoclonazepam data, as the parent drug commonly converted to its metabolite even when stored in the presence of a preservative. In instances of decreasing postmortem drug concentrations the effect of refrigerated storage could not be ruled out. A stability experiment, which contained codeine, produced data that indicated concentrations could continue to decline under the current storage conditions [88]. The cumulative data gathered for this experiment was used to identify concentration trends, which subsequently aided in the development of interpretive considerations for the specific analytes examined in the study. The identification of a vital reaction is an important forensic tool to differentiate antemortem trauma from postmortem trauma. The absence of a high STIR signal and diminished T1-weighted signal with skeletal trauma supports a lack of a vital reaction and therefore is more likely a postmortem injury [89]

1.1.6. Postmortem drug redistribution.

Postmortem redistribution (PMR) refers to the changes that occur in drug concentrations after death. It involves the

sub-discipline	purposes	applications	toxigants analyzed
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redistribution of drugs into blood from solid organs such as the lungs, liver, and myocardium. The postmortem blood involves the settling of the blood in the lower, or dependent, portion of the body postmortem, causing a purplish red discoloration of the skin. When the heart stops functioning and is no longer agitating the blood, heavy red blood cells sink through the serum by action of gravity [12, 90]. The role of postmortem toxicology is significant as it can provide vital information on possible causes of death related to illicit or prescription drugs or poisons [41]. Drug levels may be altered by attempted resuscitation and changes may occur in drug levels after death that are not predictable. In post mortem toxicology the volume of distribution of drugs is very important for analysis as this volume of distribution is the volume of plasma that would be necessary to account for the total amount of drug in the patient's body, if that drug were present throughout the body at the same concentration as found in the plasma [89-91]

The blood pH and concentrations of several chemicals are also tested in a corpse to help determine the time of death of the victim, also known as the post-mortem interval [25]. These chemicals include lactic acid, hypoxanthine, uric acid, ammonia, NADH and formic acid [90]. In postmortem situation after death, the decrease in the concentration of oxygen because of the lack of blood circulation provokes a sudden switch from aerobic to anaerobic metabolism [39, 54]. There, postmortem drug redistribution refers to the processes by which the movement of drugs and other chemical poisons between tissues, organs, and body fluids takes place after death. This phenomenon is well recognized, and was first reported 25 years ago [11, 46].

The relationship between antemortem (AM) and postmortem (PM) can be considered in morphine and codeine concentrations in whole blood. In addition, the effects of antemortem to death interval as well as the postmortem interval are considered during the interpretive process. Postmortem chemistry also called necrochemistry or death chemistry, is a subdiscipline of chemistry in which the chemical structures, reactions, processes and parameters of a dead organism is investigated [82]. Post-mortem chemistry plays an important role in forensic pathology. Biochemical analyses of vitreous humor, cerebrospinal fluid, blood and urine is important in determining the cause of death or in elucidating forensic cases [56].

1.2. Some ethical issues

Testing for drugs in hair is not standard throughout the population. The darker and coarser the hair the more drug that will be found in the hair. If two people consumed the same number of drugs, the person with the darker and coarser hair will have more drug in their hair than the lighter haired person when tested. This raises issues of possible racial bias in substance tests with hair samples. A representation of the applications of forensic toxicology has been summarized in table 3.

post mortem toxicology	evaluate contributing factors, causes and manner of death	<ul style="list-style-type: none"> • suspected drug investigation or overdose cases • suspected poison or drug related death 	<ul style="list-style-type: none"> • drugs and their metabolite • methanol • carbon monoxide and gases • ethanol, toluene and volatile substances • other toxic chemicals
human performance toxicology	evaluate effect or impairment of human behaviour	<ul style="list-style-type: none"> • drug facilitated assault, drug or other crime • suspected driving under the influence of drug road traffic test 	<ul style="list-style-type: none"> • drug and their metabolite • alcohol(ethanol) and other drug and chemicals in the blood, breath and other specimens
doping control	protect the health of athletes. maintain fair competitive standards and prevent wagering fraud	<ul style="list-style-type: none"> • use of performance enhancing drugs in human and animal sport 	<ul style="list-style-type: none"> • performance enhancing drugs • banned substances such as stimulant, anabolic steroids and diuretics in blood or urine
forensic drug testing	evaluate prior use or abuse	<ul style="list-style-type: none"> • suspected consumption of controlled drugs • workplace screening • clostridium and access to young children 	<ul style="list-style-type: none"> • drugs and their metabolites in urine

Table 3. A representation of the applications of forensic toxicology [29, 47]

1.3. Other organisms

Bacteria, parasites, maggots and other organisms that may have ingested some of the subject matter may have also ingested any toxic substance within it.

1.4. Detection and Classification of drugs in Biological Samples

Detection of drugs and pharmaceuticals in biological samples is usually done by an initial screening and then a confirmation of the compound(s), which may include a quantitation of the compound(s). The screening and confirmation are usually, but not necessarily, done with different analytical methods. Every analytical method used

in forensic toxicology should be carefully tested by performing a validation of the method to ensure correct and indisputable results at all times. A testing laboratory involved in forensic toxicology should adhere to a quality programme to ensure the best possible results and safety of any individual [66]. The choice of method for testing is highly dependent on what kind of substance one expects to find and the material on which the testing is performed. Biological samples are more complex to analyze because of factors such as the matrix effect and the metabolism and conjugation of the target compounds [7, 80].

1.4.1. Detection of Drugs

Detection of drugs and pharmaceuticals in biological samples is usually done by an initial screening and then a confirmation of compounds. Include a quantitation of the compounds. The screening and confirmation are usually, but not necessarily, done with different analytical methods. The choice of methods for testing is highly dependent on what kind of substance one expects to find and the material on which the testing is performed. Biological samples are more complex to analyze because of factors such as matrix effect and the metabolism and conjugation of the target compounds [23, 80].

1.4.2. Analysis and Profiles

The urine and blood samples that are taken during testing may be analyzed exclusively by a WADA-accredited laboratory. The Swiss Laboratory for Doping Analyses (LAD) is one such laboratory, to which Antidoping Switzerland outsources the analysis of most of the samples it collects. When the samples arrive, the laboratory checks their integrity, and then analyzes them according to the detailed standards laid down by WADA. It never knows the identity of the person from whom the sample was taken [82].

1.4.3. Direct Proof

In the laboratory, the urine and blood samples are tested for prohibited substances using a variety of analysis methods. An adverse analytical finding (AAF) is also known colloquially as a positive test. A finding is deemed adverse if it proves the presence of one or more prohibited substances (as per the Prohibited List), or their metabolites [56, 72]. The applicable regulations allow Anti-Doping Organizations to store Doping Samples for up to 10 years and to reanalyze them at a later date using refined or novel procedures.

1.4.4. Indirect Proof (Athlete Biological Passport)

In addition to direct proof of prohibited substances in the urine or blood, evidence of a violation of anti-doping regulations can be proven indirectly. To this end, certain parameters are recorded in long-term profiles, known as athlete biological passports. Observing these parameters (blood, steroid, and endocrine profile) over time reveals any unnatural changes, as well as when individual thresholds are exceeded [45, 69]. These observations are used to schedule targeted doping controls, specific follow-up analyses or for investigative purposes, and also as a basis for sanction under Article 2.2 of the Doping Statute, even if no sample has actually been found to be positive. To date, this approach has resulted in a number of athletes being successfully convicted of doping, in Switzerland as in other countries [23, 85].

1.4.5. Gas chromatography-mass spectrometry]

Gas chromatography-mass spectrometry (GS-MS) is a widely used analytical technique for the detection of volatile compounds. Ionization techniques most frequently used in forensic toxicology include electron ionization (EI) or chemical ionization (CI), with EI being preferred in forensic analysis due to its detailed mass spectra and its large library of spectra. However, chemical ionization can provide greater sensitivity for certain compounds that have high electron affinity functional groups [8, 29].

1.4.6. Liquid chromatography-mass spectrometry]

Liquid chromatography-mass spectrometry (LC-MS) has the capability to analyze compounds that are polar and less volatile. Derivatization is not required for these analytes as it would be in GC-MS, which simplifies sample preparation. As an alternative to immunoassay screening which generally requires confirmation with another technique, LC-MS offers greater selectivity and sensitivity. This subsequently reduces the possibility of a false negative result that has been recorded in immunoassay drug screening with synthetic cathinones and cannabinoids [12]. A disadvantage of LC-MS on comparison to other analytical techniques such as GC-MS, is the high instrumentation cost. However, recent advances in LC-MS have led to higher resolution and sensitivity which assists in the evaluation of spectra to identify forensic analytes [15].

1.5. Detection of Metals

The compounds suspected of containing a metal are traditionally analyzed by the destruction of the organic matrix by chemical or thermal oxidation. This leaves the metal to be identified and quantified in the inorganic residue, and it can be detected using such methods as the Reinsch test, emission spectroscopy or X-ray diffraction. Unfortunately, while this identifies the metals present it removes the original compound, and so hinders efforts to determine what may have been ingested. The toxic effects of various metallic compounds can vary considerably [45].

1.5.1. Nonvolatile organic substances

Drugs, both prescribed and illicit, pesticides, natural products, pollutants and industrial compounds are some of the most common nonvolatile compounds encountered. Screening methods include thin-layer chromatography, gas liquid chromatography and immunoassay [16, 48]. For complete legal identification, a second confirmatory test is usually also required. The trend today is to use liquid chromatography tandem mass spectrometry, preceded with sample workup as liquid-liquid extraction or solid phase extraction. Older methods include: spot test, typically the Marquis Reagents, Mecke Reagent, and Froehde's Reagent for opiates, Marquis Reagent and Simon's reagent for amphetamines, methamphetamine and other analogs, like, the Scott's test for cocaine, and the modified Duquenois reagent for marijuana and other cannabinoids. For compounds that do not have a common spot test, like benzodiazepines, another test may be used, typically mass spectrometry or spectrophotometry [71, 88].

1.6. Toxicology Discipline

1.6.1. Scope of Analysis

The Toxicology Discipline conducts *qualitative* and *quantitative* analyses. *Qualitative* analyses are used to identify a particular substance in a specimen but not to determine how much of the substance is present. *Quantitative* analyses are used to both identify a particular substance and to establish how much is present [30, 44, 78]. Quantitative analyses are more complicated than are qualitative analyses because the procedures must be calibrated and controlled for each particular drug. Because of this added complexity and for the sake of timeliness, the Toxicology Discipline may report qualitative findings without quantification or it may limit analyses to specimens where such findings can be reasonably interpreted. Quite often, the mere presence of a substance in a specimen is sufficient to answer the question whether it was consumed [83]

The Toxicology Discipline routinely conducts quantitative analyses for ethanol (alcohol) and related volatiles such as acetone, methanol and isopropanol. Headspace/gas chromatography (HS/GC) is employed for this purpose. This method may be applied to both liquid (blood, urine, etc.) and non-liquid (tissues, etc.) specimens and is specifically calibrated and controlled for these volatile substances. Non-biological specimens such as beverages may be analyzed as well. The Toxicology Discipline confirms positive findings by repeat analysis with the same or another specimen collected from the same subject [14, 39].

The Toxicology Discipline also routinely conducts qualitative analyses for prescribed and non-prescribed drugs. Some of the many drugs and drug classes detected during these analyses include the sympathomimetic amine class (amphetamine, methamphetamine, MDMA, ephedrine, etc.), cocaine and metabolites, opiate class (morphine, codeine, hydrocodone, oxycodone, etc.), benzodiazepine class (diazepam, alprazolam, etc.), barbiturate class (phenobarbital, butalbital, etc.), cannabinoids (from marijuana), other analgesics (propoxyphene, methadone, tramadol, etc.), antidepressants (amitriptyline, Prozac®, etc.), muscle relaxants and sedatives (Soma®, zolpidem, etc.). The Toxicology Discipline also routinely conducts analyses for carbon monoxide (as carboxyhemoglobin). [57, 84]. This list is not intended to be complete, rather it represents only a small sampling of the substances that comprise the scope of analysis. Please contact a laboratory for more specific information. Because toxicological findings are used in Court, the analyses are conducted according to legally defensible practices and methods. All

submitted specimens are maintained under chain-of-custody and in a secure environment to preserve the material for the intended analyses. Analyses are conducted in two phases. The *presumptive* phase is intended to provide indications whether a particular drug or drug class is present [14, 65, 86]. Specimens that appear to be presumptively positive are then subjected to the *confirmation* phase, which is intended to provide more definitive identification of the drug. Confirmation analyses are typically conducted with methods that have greater specificity than do presumptive analyses and which may examine the chemical structure of the substance as a means of identifying it. Gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC-MRM MS) are routinely employed for this purpose [9, 21]. If a specimen is initially subjected to analysis with GC/MS or LC-MRM MS, a confirmatory analysis may be conducted by repeat analysis with another specimen collected from the same subject. Positive drug findings are reported *only* when they are confirmed.

2.0. INTERPRETATION OF TOXICOLOGICAL RESULTS

2.1. Toxicological Analysis.

Toxicological analyses represent a tool for assessing the degree of impairment exerted by a drug or combination of drugs on a consumer. Because the ultimate degree of impairment is death, toxicological findings are used as part of the determination of cause and manner of death. In this light, the presence of one or more drugs at concentrations significantly higher than those expected following therapeutic doses may be considered, along with other anatomical conditions or defects, in determining whether drug consumption caused or contributed to death. Conversely, the lack of adequate drug in a subject may indicate non-compliance to therapy and allow for the conclusion that death was due to conditions that might otherwise have been survivable [23, 41, 62].

Toxicological findings may be used to assess lesser degrees of impairment such as the performance of an individual pursuant to criminal activity. A common application is to determine whether an individual has been driving under-the-influence of ethanol (alcohol, DUI) and/or drugs (DUID). Toxicological findings may also be used to determine whether the actions, behavior or demeanor of a homicide victim or suspect were affected at the time of the incident and, thereby, offer potential mitigating circumstances in adjudication of the crime. [77]. In order to establish impairment from toxicological findings, such findings must include a relevant substance present within a relevant

specimen. The reason for this is that drugs exert their pharmacological effects when they are present in a target organ or organ system.

A relevant (active) substance is one that exerts pharmacological activity and parent drugs typically fit this description. However, once ingested, normal biochemical processes within the body act upon and convert parent drugs into metabolites, which may or may not exert the same pharmacological activity as the parent drug. Furthermore, metabolites may co-exist with the parent drug or they may persist after the parent drug has been converted or eliminated [51, 91]. Consequently, only parent drug and active metabolites are relevant substances for establishing impairment. That is not to say that the identification of inactive metabolites is without value. Such may allow for, among other things, the distinction between acute and chronic drug ingestion.

The most significant target organs of forensic toxicological interest are the brain and nerves that comprise the central and peripheral nervous systems. Blood circulates within the nervous systems and as such, is the most recognized, relevant specimen. Urine, which is commonly subjected to toxicological analyses, does not circulate within the nervous systems and, therefore, does not qualify as a relevant specimen with which to establish impairment from a toxicological finding [47, 51]. Nevertheless, a toxicological finding with urine may be used to establish consumption and, depending upon the individual case and other evidence at hand, may be used to explain certain observed behavior. Toxicological findings in tissues may be considered in postmortem cases where sufficient medical literature exists to relate such findings to death or impairment.

The effects of ethanol and drugs are continuous and progressive, which means generally, the greater the dose, the greater the effect. However, the progression of effects differs amongst individuals because overall impairment is the collection of all the mental and physical functional responses to ethanol and drugs which are not uniform [31, 67]. For example, with depressant drugs, low doses will impair one's inhibitions, which results in outwardly and visibly excited behavior. However, greater doses overcome this excitation and result in lethargy, sleepiness, coma and death from respiratory depression. The point where one subject appears excited versus lethargic or lethargic versus comatose depends upon the collective degree to which all of the subject's mental and physical functions are affected [11, 20 73]. This difference is the result of individual biological characteristics and tolerance (the resistance to *some* effects and the physiological dependency on continued doses acquired through frequent and repeated exposure to

ethanol and/or drugs). Therefore, while it may be said that people respond differently to drugs (and ethanol), the truth is that people respond the same but to different degrees [2, 46].

There is a well-documented direct relationship between one's blood ethanol concentration and one's ability to operate a motor vehicle. Such relationship is reflected in the DUI *per se* laws. With drugs, there is also a well-documented direct relationship between one's blood drug concentration and the effect on one's task performance. However, because the degree of effect amongst individuals is so much more varied with drugs than with ethanol, definitive *per se* limitations are more difficult to establish scientifically and to apply legally. Some jurisdictions have zero-tolerance statutes for DUID whereas others employ the expertise of a toxicologist in Court to reconcile performance impairment with the presence a drug or combination of drugs [81, 88, 90].

2.2. Submission of Specimens for Forensic Analysis

Specimens may be submitted to the Toxicology Discipline in person or by common carrier such as the US Postal Service, UPS, FedEx, etc. The Department distributes kits, which contain the appropriate tubes, needles, seals, documentation, instructions and address labels for this purpose. There are two types of kits, one for collecting specimens from live subjects and a second for postmortem specimens and is intended for Coroners [60]. Individual specimens should be labeled with the name of the subject/suspect and packages should be sealed and initiated. Submission documentation should be sufficiently completed to allow the laboratory to determine the nature of the case and the most appropriate examinations to conduct. Completed documentation should be inserted into the pouch affixed to the outside of the kit. Kits should be sealed and initialed then submitted to the laboratory [11, 62].

The antemortem kit is intended for collection of specimens from live subjects and is appropriate for submission of specimens in DUI investigations. This kit contains two 10-mL gray-stopper blood collection tubes and one 100-mL plastic urine cup. The kit also contains a needle, alcohol-free cleansing wipes and seals. The tubes contain sodium fluoride preservative and potassium oxalate anticoagulant [1, 59] Two completely-filled tubes are recommended for a complete scope of analyses. Urine collection is optional, but highly recommended, especially when gammahydroxybutyrate (GHB) is suspected or there is a delay of more than several hours in collecting the specimens. ntemortem (DUI) kits bear an expiration date which is intended to relate to the vacuum within the blood collection tubes. Use of expired tubes will not invalidate findings determined with these specimens; however,

vacuum within the tubes may be reduced, which may reduce the volume of specimen collected and, accordingly, limit the scope of analyses which may be conducted. It is recommended that expired tubes be replaced with similar tubes from hospital stock. Kits are so marked [82, 87]. The forensic toxicology sample collection processes and analytical kits are illustrated in figure 4.

		
<p>Image 1. Antemortem (DUI) Kit</p>	<p>Image 2. Antemortem (DUI) kit contents</p>	<p>Image 3. Properly packaged antemortem (DUI) Kit</p>

UNDER REVIEW



Image 4. Properly sealed blood tube

Image 5. Low volume specimen

Image 6. Properly sealed kit



Image 7. Postmortem kit contents

Image 8. Postmortem kit needle

Image 9. Postmortem kit containers

	and syringe	
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Figure 4. Forensic toxicology sample collection processes.

2.3. Properly sealed postmortem specimens

A postmortem kit is intended for Coroners for collection of specimens from deceased subjects. This kit contains two 30-mL screw-cap containers, one for blood and the other for urine. The kit also contains a collection needle and syringe and seals. Both containers should be completely filled with the respective specimens for a complete scope of analyses. By submitting specimens, the investigating agency acknowledges that the Toxicology Discipline will conduct examinations at its discretion pursuant to current policies, procedures and capabilities and the nature of the case as determined from information provided by the submitter. The investigating agency further acknowledges that specimen volume, condition and relevance may limit the number or types of examinations which may be conducted and that whereas the Toxicology Discipline and the Department will exercise all due diligence to preserve submitted materials, such are biological in nature and, therefore, perishable

2.4. Target Toxicological Analysis

This may involve target analysis which confirms or excludes an expected target substance or a few common drugs. The circumstances of a case may require a systematic analysis for a longer list of potential toxicants and their metabolites. Owing to the wide variations in physical and chemical properties of xenobiotics and their matrices (blood, urine, etc), there is no universal drug or chemical screen. Qualitative analysis detects the presence of a substance in a sample, whereas quantitative analysis determines the concentration of the substance [78, 89].

2.4.1. Collection of information and specimens; Ante mortem specimens include blood (whole blood, plasma or serum), urine and gastric contents. Postmortem specimens from autopsies include body tissues such as liver, kidney, lung, and fluids such as blood, vitreous humour, gastric contents and bile. In some death investigations, bone marrow, skeletal muscle, hair and even maggots can be useful. Pills, capsules, tablets, powders, liquids and suspicious bottles found with the patient may also be submitted as exhibits. Specimens should be promptly collected, and properly labelled, sealed, and stored as soon as practicable, to ensure their integrity and to minimize degradation [82, 85].

2.4.2. Sampling and extraction: Homogeneity and proper sampling procedure are essential in toxicological analysis. What is sampled must be representative of the total biological specimen. Sample preparation is a critical step in the analysis, as instrumental analysis usually cannot be performed directly on biological specimens [66].

1. Headspace extraction is commonly used to identify and quantitate alcohol and other volatile compounds.
2. Liquid-liquid extraction and solid-phase extraction are commonly used for the isolation of acidic, neutral and basic drugs and other toxicants from blood, urine, and other biological specimens.
3. Sample preparation and analyte clean-up usually involve a combination of processes such as mixing, digestion, precipitation, filtration, dilution, dissolution, pH adjustment, solvent extraction and evaporation.

For most drugs and toxicants, specimens undergo a two-stage analytical process involving screening tests, followed by confirmatory tests [41, 66].

2.4.3. Screening tests: such as colour tests, immunoassays, spectrophotometry and thin layer chromatography (“TLC”) are useful for detecting the presence or absence of a particular class of compounds in a biological specimen. Breath alcohol tests are another form of screening which allows law enforcement officers to determine action steps for persons who appear to be intoxicated. These routine tests are generally rapid, require minimal sample preparation, have high throughput, and can filter out negative results quickly. Positive results from these tests suffer from the drawback that they are usually not specific or conclusive; they are considered presumptive and must be confirmed before reporting [31, 90].

2.4.4. Confirmatory tests: The detection of a chemical substance by non-specific tests must be confirmed by a second more specific technique based on a different chemical principle. These definitive techniques provide full or complementary information enabling the unequivocal identification and if necessary, quantification of the chemical substance. Modern instrumental techniques such as HPLC (high-performance liquid chromatography), gas chromatography combined with mass spectrometry (GC-MS) can be used to identify and quantify the poison available in trace amounts.

3.0. APPLICATION OF FORENSIC TOXICOLOGY.

3.1. Death investigation toxicology (Postmortem toxicology):

Postmortem forensic toxicology involves analyzing body fluids and organs from death cases and interpreting that information. Sudden unexpected and/or unexplained deaths become coroner’s cases or fall under the jurisdiction of the medical examiner. Forensic toxicologists work with pathologists, medical examiners in helping to establish the role of alcohol, drugs and poisons in the causation of death [45, 71].

1. The toxicologist identifies and quantifies the presence of drugs and chemicals in blood and tissue samples. This is done using state of the art chemical and biomedical instrumentation capable of detecting small amounts of toxic materials, positively identifying them, and accurately measuring how much is present.
2. Accuracy, validity and reliability are essential, as this information is used in the determination of cause and manner of death.
3. Accurately establishing the appropriate cause and manner of death has serious implications for public health and public safety, and forensically reliable toxicology is an essential component of that process. Death investigation toxicology is performed by both public and private laboratories and many private forensic laboratories provide specialized expertise and services not available in government laboratories.

3.2. Human performance toxicology.

Human Performance Toxicology deals with the effects of alcohol and drugs on human performance and behavior, and the medico-legal consequences of drug and alcohol use. This may include investigations of impaired driving, vehicular assault and homicide, drug facilitated crimes including sexual assault, and aircraft, motor vehicle and maritime collision investigations. It can be referred to as behavioral toxicology [14, 80].

1. Forensic toxicologists perform analysis of drugs and alcohol in biological samples, typically blood and urine, but increasingly in other matrices such as oral fluid, and hair, for the purposes of determining the timing, extent, and impairment resulting from different patterns of drug and alcohol use. The toxicologist uses those analytical methods that are found in many research and hospital laboratories to isolate drugs from complex biological samples, prepare them for analysis through extraction and purification, then determine the identity and amount of drug present.
2. This can include performance enhancement which occurs following the use of stimulants, and impairment from recreational or prescription medication use and misuse.

- Many blood alcohol concentration and drug testing cases are performed in accredited private or academic forensic toxicology laboratories. Forensic toxicologists frequently testify in court to both their findings and to their interpretation. This type of testing may occur in public crime laboratories, but also may be a function of a health department in some states.

3.3. Blood alcohol concentration and correlation with driving dysfunction has been illustrated in table 4

Drug alcohol concentration is important to control in order to ascertain driving offences and to enhance road safety policies. Breath testers that operate on the principle of infra red (IR) for analysis of alcohol in blood for traffic offences are becoming increasingly popular. The captured breath is exposed to IR light. The degree of the interaction of the light with the alcohol in the captured breath sample allows the instrument to measure the blood alcohol. Concentration in breath (61, 67, 83). The blood alcohol concentration and correlation with driving dysfunction is illustrated in table 2.

Table 4: Blood alcohol concentration and correlation with driving dysfunction [76]

BAC mg%MI	DYSFUNCTIONS
20	Insecurity; initial slowing down of the reaction time to a visible stimulus
30	Initial reduction in the sense of depth of field (stereo optometric)
40	Reduction in the corneal reflex; impoverished capacity to drive at a constant speed
50	Incapacity to drive in 25-30% of drivers, reduction in lateral visual perception, mild impairment of judgment
65	Initial alteration of balance
80-120	Reduction in the adaptability to darkness; impairment of ocular-motory coordination
120-200	Reduction in reaction times; initial diplopia; evident inebriation; serious disturbance to balance; inability to judge distances
200-300	Disorientation, mental confusion, diplopia, unstable walk
300-400	Incapacity of remaining stood up straight, state of bewilderment
>400	Coma, anesthesia, areflexia

4.0 DOPING CONTROL

The doping control procedure is clearly defined for all anti-doping organizations in the World Anti-Doping Agency (WADA) International Standard for Testing and Investigations (ISTI). Standardized procedures, professionally trained doping control officers (DCOs), and clearly formulated rights and obligations ensure that doping controls satisfy high quality standards. In Switzerland, testing is governed by the “Regulatory Statutes for Testing and Investigations [82]”.

Governing bodies of most competitive and intramural sports have derived rules regarding performance enhancing drug use to protect the health and welfare of the amateur and professional athletes, to maintain a fair and even competitive standard, and avoid wagering fraud. This applies to both human and animal sports and athletes. International groups such as the International Olympic Committee (IOC), the World Anti-Doping Agency (WADA), and the International Federation of Horseracing Authorities (IFHA) work to update and maintain these lists as patterns of drug use change [25, 49]. Forensic toxicologists in this field use many of the same high-performance analytical methods to detect current and historical use of banned substances, including stimulants, anabolic steroids, and diuretics. This type of testing occurs in commercial and public accredited laboratories around the world, though there is also testing of high-school, college and other athletes that occurs in private laboratories [78]

4.1. Doping Control Procedure

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4.2. Rights and Obligations During Testing

It is important that athletes are familiar with, exercise, and fulfill their rights and obligations when undergoing testing. The World Athletic Doping Association (WADA) has put in place regulatory guidelines for class of doping substances and doping testing procedure in competitive sports (25, 56]

4.3. Athletes’ Rights

- to treatment from the drug control officers (DCOs) that is as discreet and appropriate as possible. DCOs must provide official proof of identity.
- To be informed of the consequences of refusal to comply.
- To be accompanied by a person of trust.
- To engage an interpreter if necessary.
- To have the testing procedure explained and their questions answered.
- To ask if sample collection might be postponed briefly, e.g. for athlete presentations or award ceremonies, for media-related obligations, to participate in competitions taking place immediately, for initial regeneration measures, for necessary medical treatment and to obtain proof of identity.
- To choose from a selection of originally packaged testing kits, and to handle them themselves at all times.
- To the presence of a person of the same sex when providing a urine sample.
- To note remarks about the testing procedure, and especially anything that appears unusual, on the doping control form.
- To receive a copy of the signed form.

4.4. Athletes’ Obligations.

All competitive athletes at international level have the obligation;

- To undergo testing when required to do so (refusal to give a sample, or the evasion or manipulation of sample-giving, constitutes an anti-doping rule violation.
- To follow the instructions of the DCO.
- To provide proof of identity to the DCO.
- To confirm notification of selection for testing by signing the doping control form.
- To be supervised by a DCO (or chaperone) between receiving notification of selection for testing and arriving at the testing station.
- Urine sample collection under visual check.
- Careful checking of the information on the form, in particular the number of the testing kit.
- To sign the form after the test is completed.

4.5. Role of Doping Control Officers

Testing is carried out by trained specialist personnel, known as doping control officers (DCO). DCOs must present proof of identity and must adhere strictly to the applicable regulatory provisions. As part of this, they must brief athletes who are being tested on all steps of the testing procedure, and answer any questions they may have. This ensures that athletes' rights are safeguarded. While conducting the test, the DCO must respect the personal needs of the athlete as far as is permitted by the regulatory requirements and the local circumstances [13, 25].

4.6. The Testing Procedure Step by Step

A doping control involves taking urine and/or blood samples. A blood sample may be collected in addition to or independently of a urine sample. Detailed information on the procedures for urine and blood testing can be found via the following links:

4.6. Screening and Identification Procedures

Laboratories use a variety of analytical processes, divided into screening and identification procedures, to test urine and blood samples for substances relevant in the doping context. The first step is general screening, in which any inconclusive negative results are specifically followed up. A positive sample means that one or more prohibited substances (prohibited compounds or their metabolites) has/have been proven present [23, 40, 46]. The most common doping substances found world-wide are anabolic steroids, stimulants, and cannabinoids. These substances are detected primarily by means of gas chromatography and then mass spectrometry. Chromatography is used to separate individual chemical compounds from gas or liquid mixtures (gas chromatography GC, and liquid chromatography LC). They are then identified by means of mass spectrometry (MS). Each substance has a unique mass spectrum, comparable to a human fingerprint. Other processes, such as those applied in immunology, are used beyond MS to identify individual substances.

4.7. Athlete's Rights During Analysis

Athletes have the following rights as their test samples are being analyzed:

- To be notified of the results of the A sample analysis.
- To be present when the sample is split.
- If the A sample tests positive, to request within the set time frame that the B sample also be analyzed, otherwise the results of the A sample analysis will be deemed final.
- To be present when the B sample is opened and analyzed.
- To be accompanied by an expert of their choice for any laboratory analysis of the B sample.
- To request copies of the laboratory records concerning the A and B samples.

4.8. Forensic drug testing at workplace

Use of drugs by people in the workplace has significant safety and economic consequences. Consequently, in many states, workers in safety sensitive positions are prohibited from using recreational drugs or taking certain medications without a prescription. Particularly, in recent years there has been increased emphasis on testing employees to make sure that they are not using drugs while on the job [20, 77]. This testing started with workers in sensitive situations or those who worked in dangerous environments, such as police officers, locomotive engineers, pilots, etc., but has since spread to many other occupations. However, the testing has to be done through some enforcing standards (that has to be made by legislation through forensic departments) that requires pre-employment, random, and for-cause drug testing, such as following an accident or a transportation collision. The majority of workplace drug testing is not covered directly by accreditation programs hence there are numerous examples of improper procedures and conclusions that have led to the termination of employees at work places [55, 69]

CONCLUSION

Activities in the field of forensic toxicology is identified with the detection, identification and quantification of xenobiotics in biological and non-biological matter. A synopsis of such analytical phases leads to the interpretation of results through a rigorous evaluation criteria in relation to different regulatory areas. The two main areas where the analysis of biological material applies are forensic toxicology of dead and forensic toxicology of the living person. Forensic toxicology of the dead is devoted to determine the presence of xenobiotics in liquids and tissues and evaluate the possible causal role in the determination and dynamics of the death. Forensic toxicology of the living person is committed to determine the presence of xenobiotics in the biological specimen (blood, urine, air inhaled, hair etc) and in evaluating the possible causal role of incapacity and or deviations in behavior. With the main objective of providing scientifically based evidence, the complexity of all the above outlined roles of forensic toxicology entails the need for the adoption of quality assurance systems ascertainment methodologies and evaluation. Forensics also consider other substances that may affect a person's performance or behaviour and ability to make useful decisions based on regulatory guidelines and substances that are not complaint with employment regulation or classified as substances of abuse. The field of forensic toxicology also includes the measurement of alcohol, drugs and other toxic substances in the biological specimens along with the interpretation of the results in medico-legal contexts. A toxicological analysis can be done to test various kinds of samples. Forensic toxicology has a lot of contribution to make on Biosecurity of our environment.

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