Chapter 4

Forensic Toxicology

Donata Favretto, Massimo Montisci and Rossella Snenghi

Additional information is available at the end of the chapter

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1. Introduction

Forensic toxicology can be defined as the discipline of studiying under a forensic profile the pathologies induced by xenobiotics. This term comprehends all substances of exogenous origin that do not have a physiological role in the biochemical processes of the organism. Xenobiotics are poisons, drugs, drugs of abuse, toxins, pesticides, chemicals and agrochemicals, doping substances and, in part, food supplements.

The following application areas will be discussed: poisoning; alcohol, drugs and driving; workplace drug testing (WDT); doping-antidoping.

2. Poisoning

In the field of forensic toxicology, concepts of poisoning and adverse reaction are encompassed in the concept of intoxication according to a unitary criteriological vision; always oriented at resolving a fundamental query concerning the nexus between the action of one or more xenobiotics and the functional and/or morphological harm on the organism until its death. Substances considered *poisons* are those capable of causing damage even in low doses. Even drugs, and in any case all xenobiotics, can exert a poisonous action for an absolute overdose, for pharmacokinetic-pharmacodynamic synergies or for endogenous or exogenous factors.

The classification of poisons in Forensic Toxicology is traditionally dependent on the basis of chemico-physical characteristics of substances and on the consequent possibility of extractions from biological fluids with a method specific for classes. Therefore they have six groups: 1) *poisons in a gaseous or vaporous state* that when inhaled cause intoxication (carbon monoxide, hydrogen sulphide, ethylic ether, chloroform, etc.); 2) *poisons in a liquid state prone to volatility*

(benzene and derivatives, glycols, aldehydes, essential oils of some plants, etc); 3) *acids and strong bases* (hydrochloric, sulphuric and nitric acids, sodium or potassium hydrate, etc.); 4) *inorganic anions* (permanganates, chromates); 5) *metals* o salts of metals (arsenic, thallium, mercury, lead); 6) non volatile *organic compounds* of acidic, neutral or basic nature (the most of drugs of synthesis, alkaloids, narcotics, insecticides, agrochemicals etc.). It must be considered, however, that some toxins and many drugs, with a polypeptidic (for example insulin) or protein structure (for example erythropoietin), have physical and analytical chemical peculiarities that do render their extraction and analysis and also their classification more complex.

In many cases, poisoning represents an unknown event that can be demonstrated only through a balanced criteriology for obtaining a differential diagnoses, often inopportunely overlooked in the number of tests to be executed in cases of unexpected death. All of this leads to an underestimation of poisoning incidence in the field of so-called *medico-legal pathologies*. Included in this field are *drug related deaths (DRD)*, a widely investigated and delineated phenomenon in terms of incidence, prevalence and social impact. The multiplicity of pathological factors that contribute to its cause is demonstrated by a complex definition elaborated by a German study group [1], according to which DRD term groups deaths due to *"accidental or intentional overdoses, long term drug abuse, suicide associated to toxic dependency, lethal accidents influenced by the use of drugs"*. DRD is a pathological epiphenomenon reductively denominated an "overdose" or "adverse reaction", originating from variable physiopathological pharmacotoxicological and immunological mechanisms, whose genesis has not found and cannot find a comprehensive explanation in a morphological or chemical-toxicological cause, considered in isolation.

The phenomenon also assumes particular connotations in relation to the quali-quantitative heterogeneity of active drugs, the peculiarity of pre-existent psychophysical conditions, the unpredictability of pharmacotoxicological interactions from poly-drug abuse, capable of inducing a sometimes exponential increase of the risk of lethal *adverse reactions*.

Post-mortem diagnosis of acute and/or chronic intoxication is a paradigmatic example of the necessity to correlate circumstantial, clinical (documentary, anamnestic, objective), necroscopic (autoptic, histologic, immunohistochemical, microbiological, thanatochemical) and chemical toxicological data, obtained following an accurate methodological approach; the gathered data must successively be the object of an evaluative forensic toxicological criteriology. In this view the existence of standardised methodological protocols finds its reason, applied to the resolution of cases where an acute or chronic intoxication of forensic concern is suspected. The necessity of uniform autoptic procedures has been expressed in Recommendation N. 99 of the Committee of Ministers of the Council of Europe, relative to the harmonisation of the rules concerning the legal medical autopsy [2].

The circumstantial data draw on the spatio-temporal circumstances of the presumed "contact" with the toxicant; they can derive from police investigations, testimonial depositions, technical inspections, site inspection reports, etc., and are for example the insurgence of pathological phenomenon or of death following the ingestion of foods or beverages, or contact with chemical substances, or the retrieval of pharmaceutical provisions or paraphernalia in the location where a corpse is discovered.

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The clinical data refer to sanitary documentation that is examined to evidence any general or local disorder, in order to outline the objective symptoms characterizing an intoxication. In this view an appropriate toxicological evaluation needs a profound knowledge of the spectrum of biological effects of a specific chemical substance, of the intensity of individual effects dependent on the dose (*dose-effect relationship*) and on the frequency or probability of the appearance of a dose-dependent effect in a determined population (*dose-response relationship*).

The anatomopathological data are intended to reveal morphological modifications, pathognomonic and not, of the organs and systems of the deceased for suspected (acute or chronic) intoxication. In this field the medico-legal literature is lacking thorough studies, extended to all the organs and to the diverse modes of poisoning. In some cases, a macro and microscopic observation can enable identification of toxic-related harmfulness, also non-specific, apt to explain the death or direct the chemical-toxicological investigation. The examinations must include observance of cadaveric phenomena to capture changes in the normal coloration of hypostatic stains, detection of changes in rigidity and evolution of putrefactive phenomena, external examination (presence of jaundice, alterations in cutaneous appendages) and autopsy. A complete evaluation of macroscopic investigations, integrated by the optic and electronic microscope analyses, as well as by specific immunohistochemical techniques, may enable comprehension of the ethiopathogenesis and physiopathogenesis of the intoxication. In particular, it is important to examine the whole encephalon and the heart [3], as well as anatomical organs and structures sometimes undervalued, such as the carotid body, bone tissue and sexual organs. Examination of the *carotid body* provides indications as to possible alterations resulting from chronic hypoxic states and abuse of opiates [4]; *bone tissue*, as to alterations of bone marrow due to toxic or neoplastic diseases; *sexual organs*, as to testicular atrophy attributable to chronic exposure to steroid hormones; the *lymphonodal stations* to distinguish hypertrophy of an infective, inflammatory or neoplastic origin.

The anatomo-pathological findings allow, therefore, to address investigations in two directions:

a. To exclude acute intoxication as the principal cause of death when a natural organ pathology capable of inducing death by itself (e.g. acute myocardial infarction, cerebral haemorrhage from a ruptured aneurysm, pulmonary thromboembolism, etc.) is evidenced.

b. To strengthen and then confirm the hypothesis of acute lethal intoxication when there is evidence of anatomical lesions characteristic or suggestive of an acute fatal intoxication, also in light of documented clinical and laboratory findings.

The chemical-toxicological data are needed for the qualitative and quantitative demonstration of the presence of the toxicant in the biological fluids and in the tissues collected during the clinical or necroscopic ascertainment.

Retrieval of toxic substances in the living or dead body constitutes, in general, the most important criterion, often decisive to the diagnosis.

The chemical-toxicological analysis should be articulated according to two directions:

- *general unknown research*, when it arouses suspicion of intoxication, but the substance is ignored;
- *specific research*, when circumstantial and/or clinical documentary evidences consent to hypothesise an exposure to specific substances.

Autopsy sampling must include the encephalon, liver, kidneys, lungs, cardiac and peripheral blood, gastric content, bile, urine, head or body hair, and even bone. The samples collected must be stored in separated containers and filled as much as possible to minimise evaporation of volatile substances and the oxidation of drugs. The blood sample for quantitative analyses must be drawn from the femoral vein as this site is less exposed to post mortem alterations of concentrations of xenobiotics. Blood samples from different periferic sites and from the cardiac cavity, left and right, are useful for revealing the possible variation of the concentration of xenobiotics in the post-mortal period. In subjects who fall victim to fires, blood should be taken from vessels in regions spared by the fire, as the diffusion of carbon monoxide has been observed in literature. Specimens taken from different sites must be stored in different containers [5]. Urine can be taken before or during autopsy. The cerebrospinal fluid can be drawn, preferentially, by a suboccipital puncture or even aspirated from ventricles after the removal of the cranium. The gastric and intestinal content must be described in a detailed manner. Possible remains of drug tablets must be preserved in a separate container. In case subcutaneous or intravenous injection of drugs is suspected, a cutaneous sample should be taken from the site of injection and a cutaneous control sample from another region [5]. As to keratin matrices, they must preferably be natural hair cut from the posterior vertex of the head, where a lower variability of drug concentration is described [5]. The hair should be preserved at room temperature since freezing leads to a reduction of concentration. In a corpse, it is strongly advisable to take the hair sample before autopsy to avoid contamination with biological fluids, which cannot be completely removed by washings. Alternatively, sampling of pubic or axillary hairs, or nails, preferably taken from the feet, can be useful. However, nails contain lower concentrations of xenobiotics compared to hair.

In cases of corpses in an advanced state of decomposition it is necessary to collect larvae of diptera and other arthropods found on the body to perform entomotoxicological analyses, aimed at determining in the larvae the presence of xenobiotics originally present in the dead body fluids/tissues. Since living larvae rapidly metabolize the xenobiotics after removal from the corpse, they must be rapidly frozen and analysed as soon as possible. In addition, to avoid environmental contamination, the larvae must be washed before the analyses [6]. It is necessary to underline that, although larvae are useful as qualitative toxicological specimens, they seem to provide limited information of quantitive nature. Moreover the absence of xenobiotics in the larvae does not necessarily imply the absence of xenobiotics in their "food" source [6].

The toxicological analyses must encompass both qualitative and quantitative determinations, employing advanced technologies, using validated methods, foreseeing obligatory analyses of confirmation with certified reference standard of the xenobiotics identified. In general, two types of extraction procedures are foreseeable to separate and concentrate analytes from endogenous compounds: the liquid/liquid and the solid/liquid. The instrumental analyses will use advanced technologies able to separate analytes in the gaseous or liquid phase (gas

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chromatography, GC, or liquid chromatography, LC) and to identify them through specific detectors such as UV, IR, FID, fluorescence, mass spectrometry and multiple mass spectrometry (GC-MS, HPLC-MS, MS/MS, etc). Particularly, the use of high or low resolution mass spectrometry coupled with chromotographic technique is considered the gold standard of analytical techniques. It will also be necessary to determine concentration ratios of parenchyma-haematic, urinary-haematic, parent/metabolite compound, in order to perform a correct assessment of results from the historic, biological and statistical epidemiological points of view.

The laboratory of Forensic Toxicology has the primary task of assuring the accuracy of the results through processes of controls of the analytical and organizational quality. The organizational or logistic quality assurance implies the adoption of a protocol intended to preserve the chain of custody of biological specimens from the sampling, through analyses and reporting. Particular attention must be paid to the preservation procedures of the biological samples, both “in short term” (2°-4°C in the refrigerator) and "in long term" (freezer at a maximum temperature of -18°C/-20°C).

The analytical quality assurance must be achieved through the validation of methods, applying principles of selectivity, specificity, precision, accuracy and linearity. The analytical method must also be characterized by its LOD (limit of detection) and by its LOQ (limit of quantification). It is also necessary that the “cut-off “of the method be declared, an arbitrary measure that is adopted in order to discriminate between the results to be considered negative and the results to be considered positive. The cut-off is not therefore just a technical-analytical measure, but is also determined according to the specific diagnostic objective. The "purpose" of the research and the "aim" of the analytical element greatly influence and condition the choice of "cut off” values. Finally, it is important to remember that every analytical procedure must distinguish and appropriately use screening methods and methods of confirmation. The concept of the "confirmation" in Forensic Toxicology is indispensable, and the confirmation technique must necessarily be based on different analytical principals and/or chemical-physical characteristics from the screening procedure.

However, a positive or negative chemical-toxicological report is not sufficient proof to affirm or exclude a death by intoxication. In the first case, for example, one might identify an insufficient concentration of the toxin, arising from accidental contact or environmental contamination. A second possible explanation can be found, for example, in the method of analysis used (high limit of detection and intrinsic limitations of the analytical technique can give an apparently negative result), or in the transformation of the toxicant in metabolites or in its elimination from the organism.

For a further exemplification, the qualitative detection of a poison in the gastrointestinal tract is not sufficient evidence to establish that the substance was the cause of death. It is necessary to demonstrate that it has been absorbed and carried through general circulation, unto the organs where it has exercised its possibly lethal effect.

Similarly, the results of the urine tests are often of little significance in determining the physiopathological effects of the toxic substance, since they only allow proof that the toxic was

present in the victim's body some time before death. The physiopathological effects of the majority of the xenobiotics only correlates, in fact, with their blood concentration.

The laboratory plays a crucial role in the forensic toxicological diagnostic when a correct methodology is adopted in sampling procedures and the choice of specimen to be analysed, and when interpretation of the results is well integrated with the data acquired through other types of research.

Once the ascertainment is completed in all its stages, an evaluation phase is undertaken, in which the results of different types of tests must be comparatively and critically evaluated.

Identification of intoxication as a cause of medico-legal relevance can emerge in terms of certainty or probability, which is in turn distinguished in statistical and logical probability.

In toxicology there are not often general scientific laws, of universal or statistical epidemiological coverage, which make it possible to verify or rule out exposure to toxic substances as a cause of medico- legal relevance. It follows that one must usually have recourse to the process of rational credibility, according to the best science and experience, on the basis of what is known regarding the ethio-pathogenesis of disease from toxic origin. In the appendix, the characteristics of the most common drugs of abuse, responsible of acute or chronic intoxication, are shortly summarized according to The National Institute of Drugs of Abuse (NIDA).

3. Alcohol, drugs and driving

The studies of "Man-Machine Interaction" [7], evaluating the complex of actions and abilities required to drive motor vehicles and complex machinery (such as industrial), demonstrate that alcohol, drugs and medication influence the psychosensorial and psychomotor functions underlying such skills.

The effects of acute intake of **ethyl alcohol** vary depending on the levels of ethanolemia (in mg%mL o g/L) and the characteristics of the subject. Alcohol can induce sedation and reduction of anxiety, dyslalia, ataxia, impaired judgement and disinhibition. Alcohol has psycho-behavioural effects linearly correlated to its blood concentration. The 50 mg%mL limit, fixed by most driving codes as the limit for drunk driving, is not predictive of the disabling effects of lower concentrations, more evident in the adolescent and elderly population. In any case, the multiplication of risk by 3, 10 and 40 times applies when haematic concentrations exceed 80, 100 and 150 mg%mL, respectively. Driving with levels greater than 150 mg%mL substantiate the identification of alcohol abuse or dependency problems, in need of social-rehabilitative intervention. In Table 1 is a summary of the dysfunctions correlating to values of blood alcohol concentration (BAC) derived from clinical observation and studies on man-machine interaction.

Knowledge of the above described effects and the observation in a real or simulated driving test, allow for the following conclusions. Alcohol consumption determines a deterioration in one's driving ability through an increase in speed, loss of awareness, impaired visual function

and attention, wavering about the lane markings, slowing down of reaction time for "breaking and steering", overestimation of "collision" time, inadequate risk assessment [8,9]. Scarce experience, age, alcohol tolerance are factors enhancing alcohol related driving incapacity.

BAC mg%mL	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

Table 1. Values of BAC and correlation with driving dysfunctions

The central nervous system is affected not only by the acute effects of ethanol abuse, but also from chronic intake. A significant percentage of alcohol dependents are affected by dementia, cerebellar degeneration, peripheral miopathies and neuropathies.

Incapacity to drive caused by *Cannabis indica* (marijuana and hashish), varies according to the dose of active ingredient taken, not excluding the accidental consumer. Alterations of performance are reported for values of tetrahydrocannabinol (THC, the active principle of Cannabis) comprised between 2 and 5 ng/ml [10]. THC leads to an increase in systolic pressure and cardiac frequency, conjunctival hyperemia, difficulty with nocturnal vision and focusing on objects, above all if in motion, reduction in awareness, distortions of space and time, delayed reactions to stimuli, anxiety, paranoia, panic attacks, motor coordination deficit and impaired judgement with a greater propensity for "risk taking" [11]. These effects, especially expressive with speed and "wavering" of the car (as demonstrated by real driving tests), are accentuated by and also affect reaction times when cannabis is consumed in combination with alcohol [8], as well as together with other psychoactive substances. The effects of cannabinoids are also found in chronic consumers in the long term.

All **hallucinogens**, of natural origin (mescaline, psilocine, psilocybine, etc.) or of synthesis (LSD, derivatives and analogues) induce driving incapacity, seriously altering all the sensitive, neurocognitive, and psychomotor functions, at any dose, either in a state of tolerance or intolerance. The hallucinogenic effects, which start from 15 minutes to 1 hour after ingestion, determine the increase of arterial pressure and of body temperature, as well as spatial-temporal distortion and depersonalisation, often leading to suicide attempts.

Cocaine and **amphetamines** are the most prevalent disabling psycho-stimulants, which, though causing a "HIGH" stage and initial improvement of certain psycho-motory functions (reaction time, attention, awareness), cause an incapacity by altering risk perception [12,13]. The initial stimulating effects are soon substituted by tremors, hypertension, tachycardia and, in the case of amphetamines, increase in body temperature and manifestation of epileptic convulsions. In the "DOWN" phase, debilitating effects follow such as depression, irritability, fatigue and anxiety attacks. The down phase can occur a early as 45 minutes after ingestion and has a duration of 2-4 days. As for amphetamine derivatives (e.g. ecstasy) physical and behavioural effects manifest after just a few minutes of ingestion, and protract up to six hours. Such effects, due to a generalized stimulation of the central nervous system, include: euphoria, hyperexcitability, nervousness, tachycardia, insomnia, anorexia, bruxism and mydriasis. Paradoxically, such effects are accompanied by a sense of wellbeing and relaxation and some ameliorative psychological effects. Among the chronic effects are deficits in cognitive functions (memory loss, difficulty concentrating and learning) and psychotic flashbacks. For both hallucinogens and psycho-stimulants drugs, studies of man-machine interaction demonstrate: an increase in dynamic variations of the motor vehicle, both in a lateral and longitudinal (waving) direction; maintenance of high speed; notable reduction of safety distances; reduced reaction to sound and visual stimuli (mydriasis). The above-mentioned effects, indicators of a considerable risk of road crashes and accidents [14], are accentuated or favoured by the combined intake of ethylic alcohol or the increase in drug dose (lateral undulations, increase in speed and reaction times).

The role of natural **opiates**, *Methadone* and *Buprenorphine* in the determination of road accidents is still debated. The verified effects on the incapacity to drive correlate to mood changes, reduced motor coordination, drowsiness, slower psychomotor coordination and pupillary constriction. Withdrawal symptoms and frequent association with other psychoactive substances take on considerable importance, particularly with ethyl alcohol. For some authors Methadone Maintenance Treatment is thought to impede capacity to drive, until psychic stabilisation (> 1 year) and secure absence of the co-use of other psychoactive substances. Buprenorphine in healthy subjects increases reaction time in a laboratory test but not in a simulation driving test.

Studies that have evaluated all medications employed in heroine dependence therapy have shown that the parameters of competence (deviation from the lateral standard position, speed and capacity to steer round a bend, reaction to stimuli), do not present a difference in treated patients *vs* controls, except in the case of combined intake of ethylic alcohol [15].

Gamma hydroxybutyrate (GHB), a natural constituent of diverse systems and apparatus of the human organism, also a drug used in anaesthesiology therapy and in the treatment of

alcohol dependence, is the object of abuse for its euphoric, sedative and anabolic effects. Other effects deriving from GHB intake include: disorientation, slowness to react, agitation, inability to focus attention, impaired coordination and balance, tremors, drowsiness, unconsciousness. Given the capacity of GHB to induce sedation, the possibility of determining incapacity to drive motor vehicles becomes evident. It produces collateral effects characterized by nausea, vomiting, drowsiness, vertigo, bradycardia and respiratory depression, coma. Association with alcohol exponentially increases the above described effects [16].

As far as **medicinal drugs** are concerned**,** there is an *ample difference* between the effects of psychotropic *medicines* belonging to the *same therapeutic class.*

Among *Antidepressants*, *Barbiturates*, *Benzodiazepines*, *Hypnotics* and *Neuroleptics,* are disabling medicines and medicines for which the conclusions are not definitive. Also among *Anxiolotics* and *Antihistamines* coexist disabling drugs and drugs free from effects.

In particular, psychopharmacological studies of man-machine interactions (real and simulated driving test, laboratory test) have identified: drugs that induce an incapacity to drive; drugs that determine a positive effect on the capacity to drive; drugs that do not determine any effect [17].

An analytical systematic research in the field of Alcohol, Drugs and Driving poses particular problems regarding: correct blood sampling, which requires skin disinfection with an alcohol free liquid and blood drawing with a vacutainer vial containing suitable preservatives and anticoagulants; urine sampling, carried out under visual control; use of a chain of custody that documents time, place and personnel engaged in collection and transfer of samples; written consensus to sample collection and analysis given by the subject suspected of DUI, when considered by statuary laws; analytical procedures to be adopted; conservation of specimens in case of possible contestation.

Analytical procedures for the determination of BAC must be in headspace gas-chromatography with a suitable specific detector, whereas the determination of ethanol by enzymatic methods in serum or urine is not suitable for a report of medico-legal value.

The range of substances that must be determined qualitatively and quantitatively is wide, and includes both scheduled and prescription drugs (e.g. benzodiazepines, Z drugs), depending on specific Country legislation. It is thus necessary to have more validated methods available, both in GC-MS and LC-MS, and adequate reference standards. In the evaluation phase, only concentrations identified in the blood may be related to the state of impairment, although there is no univocal approach: in some Countries, threshold values are given by law (blood cut-off) to determine riving impairment, in other Countries no values are set and every concentration is considered impairing (*zero tolerance*).

For the use of oral fluid as the biological fluid for road side DUI controls, the following problems arise: absence of standardized procedure for sample taking; frequent paucity of specimen compared to conventional matrices (e.g. blood) with consequent limitations of multiclass analyses and counter analyses; greater concentration of active compounds than their metabolites, detectable at low concentrations if not sometimes absent; variability of the

relationship between salivary and blood concentrations, depending on the variability of the salivary pH, in turn dependent on the speed of saliva production; the possibility of oral contamination as a result of endonasal or inhalatory intake (smoking) of a substance, with a consequent increase of the salivary concentration, independent of the blood concentration.

In light of such criticality, analyses on saliva specimen introduce prospects of controversy connected to analytical, kinetic and evaluative problems.

4. Workplace Drug Testing (WDT)

The problem with the consumption of psychoactive substances in the workplace has been the object of multiple studies, characterized by investigations on diverse populations of workers (deceased or hurt in a work related injury or subjects recruited with random criteria or for a toxicological control duty) and different methodologies of research (survey, chemical-toxicological analyses on biological matrices, integrated approach) [18]. Despite the limitations of comparative interpretation, the results of the highlighted studies reveal that:

- cannabis is generally the most diffuse narcotic substance in workers subject to random and mandatory checks;
- alcohol, opiates and cannabis are the most frequently detected substances in injured and deceased workers subject to sporadic toxicological checks.

Given that such evidence does not patently allow for establishing the significance of the role of psychoactive substances, in the genesis of accidents at work, the need to implement systematic, international and national studies, including comparative analysis of anamnestic/cathamnestic, clinical/autoptic and chemical-toxicological data, extended to multiple biological matrices (blood, urine and hair), exists.

There are guidelines elaborated by International Scientific Societies and statuary legislations that provide instructions for authorized laboratories to perform toxicological analyses in the work place frame. Such indications regard: the method of specimen collection and analysis; interpretation of results, also dependent on predetermined cut-off statutory values; internal and external monitoring of analytical quality; the manner of reporting.

Organization of toxicological controls on workers presumes therefore the application of uniform and standardised toxicological assessment, aimed at safeguarding the security of work places, also through acquisition of scientific epidemiological evidence.

5. Doping

Doping urges for an institutional and collective attention as an underground phenomenon in rapid growth in the sporting world and social reality. Changing and refinement of substances and doping methods, their growing pharmacodynamic effectiveness, lacking or problematic

detectability of substances with ergogenic purposes (so called food supplements), widespread and clandestine commercialization of drugs, and the volume of annual trade, all signify a state of emergency.

In this context, death caused by doping is an event routinely reported by high level sporting competitions [19]. The scarcity of data deduced from international literature and the lack of institutional systems of epidemiological monitoring does not permit reliable estimates of the size of the phenomenon, even and above all with reference to amateur sporting activity. The intrinsic dangerousness of all doping practices, as well as the remarkable heterogeneity of protocols investigating an athlete's sudden death, contribute to the problem. The risk of death related to use of doping substances and practices is mainly associated to anabolics and stimulants, followed by erythropoietic stimulating drugs, growth hormones and diuretics [20].

However, paucity of epidemiological data relative to doping consumption and correlating cases of deaths, cannot define the real size of the phenomenon, which is certainly underestimated. The use of doping seems to be accepted by the sporting community, also because of the underestimation of the related dangers.

The inability to define the real danger is reflected in the failure to alert institutions, entrusted with the implementation of preventative, informative and repressive educational interventions.

To dam difficulties associated with the growing abundance of doping substances, laboratories must exchange their experiences and rational adjustment of procedures is needed in a process of constant renewal, in technology and analytical procedures. The renewal must involve use of systems of greater sensitivity and analytical accuracy, as well as larger systematic screenings to incorporate new banned substances in real time.

6. Conclusion

Activity 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 *evaluative criteriology* in relation to different regulatory areas.

The two main areas where the analysis of biological material applies are «forensic Toxicology of the 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 or concausal 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 or concausal role of incapacity and/or deviations in behaviour (see suitability to drive, WDT, doping, etc.), or rather harm to the person.

Obligation in the above-mentioned areas is complex because of «pre-analytical» and «analytical» variables. Among the pre-analytical variables are: quantity of dose ingested, frequency and means of ingestion, interval between intake and sample taking, the sample collection procedure, the interval between sample taking and analysis.

Among the analytical variables are: elevated number of analytes, large variety of chemical structures, of volatility, functional groups, hydrophilic/lipophilic ratios, values of pK_a or pK_b; wide ranges of concentration in liquids and biological tissues, dependent on dose intake; the way the specimens are stored; the possible lack of pharmacokinetic and pharmacodynamic studies; the diversity of biological matrices and potential analytical interferences produced by exogenous, endogenic and putrefactive substances.

The complexity of those variables ensures that *every analysis may be given as an individual case for which there are no rules applicable to all xenobiotics and all situations*.

With the diffusion of environmental toxins and the clandestine drug market, the forensic toxicology laboratory is also committed to the analysis of non-biological material. In this context, Forensic Toxicology can provide to institutions and society information and awareness on the appearance of new drugs; identification of the major channels of drug distribution in the local and national black market; identification of the means adopted by traffickers to bypass systems of control; information on substances used in the cutting or treatment of the drug; suggestions for timely legislative adaptations.

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, ascertainement methodologies and evaluation criteriologies.

7. Addendum

7.1. Principal drugs of abuse

7.1.1. Nicotine

Alakloid from *Nicotiana tabacum*; found in cigarettes, cigars, and smokeless tobacco (snuff, spit tobacco, chew); it can be smoked, snorted, chewed

Acute Effects - Increased blood pressure and heart rate

Health Risks - Chronic lung disease; cardiovascular disease; stroke; cancers of the mouth, pharynx, larynx, esophagus, stomach, pancreas, cervix, kidney, bladder, and acute myeloid leukemia; adverse pregnancy outcomes; addiction

7.1.2. Ethanol

Alcohol, ethyl alchol, produced by sugar fermentation; found in liquor, beer, and wine; orally ingested (swallowed)

Acute Effects - In low doses, euphoria, mild stimulation, relaxation, lowered inhibitions; in higher doses, drowsiness, slurred speech, nausea, emotional volatility, loss of coordination, visual distortions, impaired memory, sexual dysfunction, loss of consciousness

Health Risks - Increased risk of injuries, violence, fetal damage (in pregnant women); depression; neurologic deficits; hypertension; liver and heart disease; addiction; fatal overdose.

7.1.3. Tetraydrocannabinol

Active principle of *Cannabis*; found in marijuana or hashish or hash oil or hemp; smoked or swallowed.

Scheduled drug.

Acute Effects – Euphoria; relaxation; slowed reaction time; distorted sensory perception; impaired balance and coordination; increased heart rate and appetite; impaired learning, memory; anxiety; panic attacks; psychosis

Health Risks - Cough, frequent respiratory infections; possible mental health decline; addiction

7.2. Opioids

Alkaloids from Papaverum Somniferum; found in opium (Morphine, Codeine) or derived from opium by chemical synthesis (Heroin) or chemically synthesized (Naloxone, Oxycodone, Oxymorphone etc); Heroin (diacetylmorphine) can be injected, smoked, snorted; Opium can be swallowed or smoked; scheduled drugs.

Acute Effects - Euphoria; drowsiness; impaired coordination; dizziness; confusion; nausea; sedation; feeling of heaviness in the body; slowed or arrested breathing

Health Risks - Constipation; endocarditis; hepatitis; HIV; addiction; fatal overdose

7.2.1. Stimulants

Alkaloids found in *Coca* leaves (Cocaine) or chemically synthesized (amphetamines and methamphetamines, methylenedioxyamphetamines, methylenedioxyamphe-tamines, amphetamine-like compounds, phenethylamine derivatives); Cocaine can be snorted, smoked, injected; Amphetamine derivatives can be swallowed, snorted, smoked, injected; scheduled drugs.

Acute Effects - Increased heart rate, blood pressure, body temperature, metabolism; feelings of exhilaration; increased energy, mental alertness; tremors; reduced appetite; irritability; anxiety; panic; paranoia; violent behavior; psychosis. **for MDMA** - Mild hallucinogenic effects; increased tactile sensitivity; empathic feelings; lowered inhibition; anxiety; chills; sweating; teeth clenching; muscle cramping

Health Risks - Weight loss, insomnia, sleep disturbances; cardiac or cardiovascular complications; depressions; stroke; seizures; addiction. **for MDMA** - Sleep disturbances; depression; impaired memory; hyperthermia; addiction.

Also, for cocaine – Nasal damage from snorting

Also, for methamphetamine – Severe dental problems

7.2.2. Dissociative drugs

Synthetic drugs: Ketamine (injected, snorted, smoked); Phencyclidine (PC) and analogs (swallowed, smoked, injected). **Naturally occurring**: salvinorine from *Salvia divinorum* (chewed, swallowed, smoked).

Acute Effects - Feelings of being separate from one's body and environment; impaired motor function

Also, for ketamine - Analgesia; impaired memory; delirium; respiratory depression and arrest; death

Also, for PCP and analogs - Analgesia; psychosis; aggression; violence; slurred speech; loss of coordination; hallucinations

Health Risks - Anxiety; tremors; numbness; memory loss; nausea

7.2.3. Hallucinogens

Synthetic drugs*:* Lysergic acid diethylamide (LSD, swallowed, absorbed through mouth tissues).

Naturally occurring: Mescaline, from the peyote cactus (Lophophora williamsii), the San Pedro cactus (Echinopsis pachanoi) and in the Peruvian torch (Echinopsis peruviana), (swallowed, smoked); Psilocybin, from mushrooms of genus Psilocybe, such as P. azurescens, P. semilanceata, and P. cyanescens, and from about a dozen other genera (swallowed).

Acute Effects - Altered states of perception and feeling; hallucinations; nausea

Also, for LSD - Increased body temperature, heart rate, blood pressure; loss of appetite; sweating; sleeplessness; numbness, dizziness, weakness, tremors; impulsive behavior; rapid shifts in emotion

Also, for Mescaline - Increased body temperature, heart rate, blood pressure; loss of appetite; sweating; sleeplessness; numbness, dizziness, weakness, tremors; impulsive behavior; rapid shifts in emotion

Also, for Psilocybin - Nervousness; paranoia; panic

Health Risks, for LSD - Flashbacks, Hallucinogen Persisting, Perception Disorder

7.2.4. Anabolic steroids

inhalants

Acute Effects, for Anabolic steroids - No intoxication effects.

for Inhalants (varies by chemical) - Stimulation; loss of inhibition; headache; nausea or vomiting; slurred speech; loss of motor coordination; wheezing

Health Risks, for Anabolic steroids - Hypertension; blood clotting and cholesterol changes; liver cysts; hostility and aggression; acne; in adolescents—premature stoppage of growth; in males—prostate cancer, reduced sperm production, shrunken testicles, breast enlargement; in females—menstrual irregularities, development of beard and other masculine characteristics

for Inhalants - Cramps; muscle weakness; depression; memory impairment; damage to cardiovascular and nervous systems; unconsciousness; sudden death.

Author details

Donata Favretto*, Massimo Montisci and Rossella Snenghi

*Address all correspondence to: donata.favretto@unipd.it

Legal Medicine and Toxicology, University Hospital of Padova, Padova, Italy

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