FORENSIC TOXICOLOGY

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Keywords : Forensic toxicology, history, recent developments, analytical toxicology, spectrometric methods, chromatographic methods, immunoassays, quality assurance, alternative matrices, post-mortem toxicology, drug facilitated crime, drugs and driving, pharmaceutical and illicit drugs, inorganic poisons, natural toxins, toxic mushrooms, toxicological findings interpretation, future challenges.

Content

Summary

A look at history of forensic toxicology shows us that poisons were difficult to detect and legislation inadequate until progress of toxicological analysis was made in the 19th century. Many famous criminal poisoning cases contributed to progress of toxicology. In this chapter a summary of pharmacodynamic and pharmacokinetic properties of some common toxic inorganic, organic natural compounds, synthetic drugs and toxicants are outlined. The general principles of forensic toxicology followed by the tools available for post-mortem or living persons' case work are explained. The different human biological samples, food, drinks, medicines, drug addict's paraphernalia that can be investigated by the forensic toxicologist are specified. Quality assurance aspects are described. Great emphasis is given to the interpretation of toxicological findings. The

aims and achievements of scientific organizations and associations of forensic toxicology are briefly discussed. Future challenges and needs for forensic toxicology are commented.

1. Introduction

Toxicology (derived from the Greek τοξικός and λόγος) is an interdisciplinary science dealing with the study of adverse effects caused by xenobiotics on living organisms, notably study of intoxication or behavioral symptoms, action mechanisms aka pharmacodynamics or toxicodynamics (what the toxicant does to the body), disposition aka pharmacokinetics or toxicokinetics (what the body does to the toxicant) and bioanalysis, risk assessment and specific treatment of intoxicated patients. Xenobiotics are chemicals that are foreign to living systems and usually harmful, either toxins as biochemicals produced by living systems such as plants or animals or toxicants (the other toxic chemicals). Pharmacokinetics and toxicokinetics deal with the absorption, distribution, metabolism of xenobiotics on their way to target sites, and excretion from human body. Disposition depends upon the current biochemical status of the organism e.g. metabolic enzyme status at time of exposure, nutritional status, general health status, stress level, etc. Toxicological analysis deals with the detection, identification and quantification of xenobiotics in biological systems. A great deal of toxicological knowledge is either based on experimental animal *in vivo* toxicity testing, *in vitro* toxicity testing or on epidemiological studies in humans at occasional poisoning cases. As for ethical reasons intoxication experiments cannot be made with humans, publication of observed incidental poisoning cases is therefore essential. It is now well recognized that virtually all toxic effects are caused by changes in specific cellular molecular targets.

Forensic toxicology deals with the investigations of causes of death, poisoning and inadequate or recreational drug use. Forensic toxicology uses notably analytical chemistry, pharmacology/toxicology and clinical chemistry for these investigations and is mainly focused on the interpretation of the obtained toxicological findings. Forensic toxicologists are specialized scientists basically trained as biologists, chemists, pharmacists, physicians, veterinarians or several of these, who investigate any suspect symptoms recorded, any evidence collected at the scene and determine a concentration or dose/response relationship of the xenobiotic in an individual related to the cause of death, behavioral changes or impairment.

2. History of Forensic Toxicology

Antiquity

History of toxicology begins about 50 000 BCE with early humans who used poisonous plants and animals for hunting or as warfare agents. By 1500 BCE, written evidence indicated that hemlock, opium, curare, and certain metals were used for poisoning. In antiquity such as in the Sumerian-, Median-, Assyrian-, Persian-, Egyptian-, Indian- aka Ayurvedic-, pre-Columbian-, Chinese-, Hellenistic-, and Roman- medicine knowledge slowly improved, but was guided by mythology, religious believes and rituals, folklore,

shamanism, philosophy rather than by science with observation, experiments and theories.

Hellenistic medicine from Hippocrates (ca460-ca370BCE) its founder, who first described many diseases including poisoning with fungi, herbs, plants and foods, via Aristotle (384-322BCE), who published "*De historia animalium"*, Theophrastus (ca371-287 BCE) Plato's student and Aristotle's successor at the Lyceum, documented the properties of more than 300 plants in "*De historia plantarum"* serving as a reference in botany for many centuries, Nicander of Colophon (204-135BCE), to Mithridates VI Eupator (132-63BCE), King of Pontus (today Turkey) who developed a so-called "universal antidote" with 36 ingredients named "*mithridatium"*, offering in fact little protection, foundation of toxicological knowledge was initiated.

In 82 BCE Roman Dictator Lucius Cornelius Sulla Felix (ca 138-78BCE) passed the first known law against criminal poisoning called *Lex Cornelia de Maiestate*. Cleopatra (69-30BCE), the last Pharaoh of Ancient Egypt, member of the Ptolemaic dynasty, tested toxicity notably of strychnine on prisoners and slaves. Marcus Fabius Quintilianus (35-96CE) a Roman rhetorician from Hispania declared at court to the judges: "*Credite mihi judices, difficilius est venenum invenire quam inimicum"*, Andromachus father (1st Century CE), personal physician of Emperor Nero Claudius Caesar Augustus Germanicus (37 - 68CE) with great interest in poison, "improved "Mithridates' antidote recipe into a concoction containing 64 ingredients named "*Theriaca andromachi"*. Pedanius Dioscorides (ca40-90 CE) a Greek herbalist and practicing physician also at court of Emperor Nero explained in 70CE the therapeutic use in "*De materia medica*", a precursor of modern pharmacopoeias with comprehensive descriptions of some 600 plants in addition to Nicander's *Theriaka*, notably snake bites, mandrake, etc. Wolfsbane *Aconitum napellus* was so frequently used for killing, that Emperor Marcus Ulpius Nerva Trajanus Augustus (53-117CE) banned its growing in all Roman domestic gardens. Claudius Galenus of Pergamon (129-199/217CE) made major and fundamental contributions to science such as anatomy, physiology, pathology, neurology and pharmacology/toxicology including antidotes.

Middle Ages and Renaissance

By 711CE the Saracens who had conquered Alexandria in 640CE, translated the Greek texts related to medicinal herbs into Syrian and spread this knowledge all over the Orient. This knowledge from Greco-Roman tradition, Jewish-Arabic, Persian, Middle East and northern Africa practices and experience from Oriental scholars, notably Muhammad ibn Zakariyā Rāzī (865-930 CE), Avicenna aka ibn Sina (980-1037 CE), Maimonides aka Rabbi Moshe Ben Maimon Mūsā ibn Maymūn (1138 - 1204 CE), and Al Nabarawi (d 1126 CE) was preserved after the fall of Rome in 410 CE, throughout the Crusades (11th to 13th Century CE), and re-entered Europe principally via Salerno - a geographic, political, and philosophical crossroad renowned as *Hippocratia civitas* with its *Scuola Medica Salernitana.* During the Early Medieval Period medical studies and practice were widespread in western monastic circles, where monks and nuns collected medicinal recipes, and cultivated herbs. Bartolomeo da Varignana (1255- 1321CE) performed in 1302 CE the first autopsy in Bologna to investigate a poisoning case,

according to a 1209CE decision by Pope Innocent III (1160-1216 CE) and in 1234CE by Gregory IX (1170-1241CE) to anchor medico-legal expert opinion in the *Corpus Iuris Canonici,* in case of suspect death. Paracelsus (1493-1541CE) formulated in 1538CE a fundamental cited version in Latin from the 18th century *"Dosis sola facit venenum"*, but originally written in old German: *Alle Ding' sind Gift, nichts ohn' Gift, allein die Dosis macht dass ein Ding kein Gift ist.* The 16th century pioneer anatomists and physicians Andreas Vesalius (1514-1564CE), Paolo Zachia (1584-1659 CE) and Ambroise Paré (1610-1590CE), who performed the first forensic autopsy in France in 1562 CE, made enormous progress in medical knowledge, allowing also toxicology to improve. The famous inscription in many morgues worldwide *Mortui vivos docent* is attributed to Giovanni Battista Morgagni (1682 -1771CE) from his textbook *De sedibus et causis morborum per anatomen indagatis* published in 1761CE. In this book article 21 *De morbis a venenis inductis* he wrote: *Sed res certa erit,ubi in ventriculo,aut proximis intestinis venenum ipsum reperietur facile agnoscendum,* and was probably the first to point out that diagnosis of poisoning can only be made by detection of the suspected poison.

18th Century CE

In 1758 a statement*"Alimenta a toxis, uti medicamenta a venenis, non natura sed dosis distinguit"* similar to the statement of Paracelsus was formulated by Carolus Linnaeus (1707-1778), the founder of modern systematic fauna and flora studies. In a poisoning trial at Oxford in 1752, the first known chemical test ever for arsenic detection was performed by medical examiner Anthony Addington (1713-1790).

19th Century

In a transition period of the 19th century, where medical mindset between natural philosophy and natural sciences changed, an enormous progress of toxicological analysis was made. First a more reliable arsenic detection and quantification method was developed 1836 in UK by James Marsh (1789-1846). In Paris pioneer toxicologist Mateo José Bonaventura Orfila (1787-1853), a Spanish native, considered as the father of modern toxicology first established a systematic correlation between a chemical and its biological effects and could demonstrate associated tissue damage in specific organs by analyzing autopsy material. The work of Orfila was continued in the UK notably by his students Robert Christison (1797-1882) at Edinburgh University, Alfred Swaine Taylor (1806-1880) at Guy's Hospital, London and in Sweden by Jöns Jacob Berzelius (1779-1848) at Karolinska Institute, Stockholm. Other toxicologists notably Thomas Stevenson (1838-1908) followed at Guy's Hospital and became involved in many famous trials for criminal poisoning. Pioneers in inorganic toxicology were Hugo Emil Reinsch (1809-1884) using copper wire in to detect Hg, As, Bi, Sb in biological samples in 1841, or Carl Remigius Fresenius (1818-1897) and Lambert Heinrich von Babo (1818-1899) who in 1845 respectively in 1847 proposed screening methods for mineral poisons based on Justus von Liebig's (1803-1873) concepts. Jean-Servais Stas (1813-1891), professor at the Royal Military Academy in Brussels, another pioneer, was the first in 1851 to perform the isolation of alkaloids notably nicotine from alkalinized biological specimens by solvent extraction using diethyl ether after deproteination with ethanol for convicting the poisoner in the famous Bocarmé case. This isolation method

was modified in 1856 by Friedrich Julius Otto (1809-1870) to a systematic method for the extraction of non-volatile acidic and alkaline compounds from biological tissues. In 1859 Robert Bunsen (1911-1899) and Gustaf Kirchhoff (1824-1887) at Heidelberg University discovered flame emission spectrometry to identify and quantify mineral elements. After the first test for alkaloids by Ferdinand Ludwig Winckler (1801-1868) in 1830 using chemical reagents with mercury and potassium salts many subsequent developments of color or precipitation tests for alkaloids identification were described, notably by German-born pharmacist Johann Georg Draggendorff (1836-1898) at the University of Dorpat (nowadays Tartu in Estonia) and his assistants Eduard Marquis and Karl Friedrich Mandelin (1854-1906). Subsequent microscopic examination of obtained crystals or subsequent melting point determination was routinely performed until the 1960s. Pure liquids could be identified by their refractive indices. Before these chemical tests became available, toxicologists had to identify poisons by typical odors, by taste or by physiological tests, notably the tetanic spasms test in frogs (1856) for strychnine detection; the mydriasis test of cat eyes proposed by the German chemist Friedlieb Ferdinand Runge (1795-1867) for atropine detection; the taste test giving peculiar numbness of the tongue with aconitine - the Queen of poisons, one of the most potent plant derived toxin; the Straub-Hermann mouse tail test by Walther Straub (1874-1944) for morphine administered subcutaneously inducing a characteristic S-shaped mouse tail; and the asystole observed on isolated frog heart by Rudolf Böhm (1844-1926), and Claude Bernard (1813-1878) for digitalis detection in 1856. Francesco Selmi (1817-1881) began as the first to doubt the reliability of color reactions for forensic toxicological purposes, because of interference with physiological components of various organs, such as ptomains obtained by post-mortem degradation.

20th Century

In 1924 Louis Lewin (1850-1929) considered as the father of modern psychopharmacology, published a pharmacological classification of psychotropic drugs into: euphorica, phantastica, inebriantia, hypnotica, and exitantia useful to understand toxicity and published several other papers on hallucinogenic plants. Practical laboratory glassware required for micro-diffusion methods to detect volatile compounds such as hydrogen cyanide, carbon monoxide, etc were introduced 1947 by Edward J Conway (1894-1965). From 1920 to present time important discoveries in molecular pharmacology describing the role of chemical transmitters in nervous system such as acetylcholine, dopamine, noradrenaline, GABA, neuropeptides (endorphins, enkephalins), second messengers such as cAMP, cell membrane and nuclear membrane receptors, signalling proteins, G protein-coupled receptor kinases, ion channels, etc were made, allowing progress to understand action mechanisms of psychotropic substances. In 1957 psychiatrist Jean Delay (1907-1987) at Sainte Anne Hospital in Paris and his assistant Pierre Deniker (1917-1998) proposed a new pharmacological classification of psychotropic drugs into psycho-analeptics (notably stimulants and antidepressants), psycho-dysleptics (notably hallucinogens, narcotics and alcohol), and psycholeptics (hypno-sedatives, Li salts, neuroleptics, anticonvulsants) validated at the 1961 World Congress of Psychiatry in Paris. This classification was up-dated several times since that date. Biological assays, using the vinegar fly *Drosophila melanogaster* to screen for insecticides in body tissues were proposed in the 1960s.

Evolution in Ethanol Research

Systematic research of EtOH pharmacokinetics and BAC determination was developed in 1922 by Erik Widmark (1889-1945). The Drunk-o-meter to measure BrAC was invented by Rolla Harger (1890-1983) in 1938. An enzymatic ADH method for EtOH determination was introduced by Roger Bonnichsen (1913-1986) in 1951. The Dräger presumptive test for EtOH became available in 1954. The Intoxymeter invented 1954 by Robert Borkenstein (1912-2002) to estimate the BrAC, became later the Breathalyzer. A determination method for EtOH and minor ingredients in alcoholic beverages with head space gas chromatography was proposed by Gottfried Machata (b1925) and Wolfgang Bonte (1939-2000).

More Recent Developments in Analytical Toxicology

As shown in Table 1 and Table 2 development of new extraction techniques, chromatographic and spectrographic technologies mushrooming all over the scientific world since the 1960's, analytical toxicology made tremendous methodological improvements.

Since Goldbaum's method for barbiturates spectrometric methods in the 1950s, great progress was made. Another revolution in analytical toxicology was pioneered at Karolinska Institute in Sweden with Ryhage's application of GC-MS to toxicology, further developed by many others, notably the Pfleger-Maurer group from Homburg/Saar in Germany. Paper chromatography, thin layer chromatography(TLC), gas chromatography (GC) and high performance liquid chromatography aka high pressure liquid chromatography (HPLC) one of the most performing separation technologies was followed by automatization to run series of many specimens, and by LC-MS and capillary electrophoresis in the 1970s, later applied to toxicology by Franco Tagliaro from Verona in Italy. With the emerging of immunoassays for insulin in the 1950's and subsequent development for drugs-of-abuse testing, full automatization of these tests was developed. After this evolution a Lab-on-a-chip has become available integrating one or several laboratory functions on a single small chip allowing the handling of less than 1 pL e.g a multiplex biochip array technology marketed by Randox Company allowing multi-analyte drug screening or other diagnostic parameters in biological samples adaptable to the local geographic situation. In the 1955 to 1965 period LODs could be decreased from mg to µg range and in 1965 to 1975 period from µg to ng range and later even below.

Years	Analytical Technologies
< 1960	Colour and Microcrystal Tests
	Thermal Microscopy, Melting Points, Paper Chromatography
1960	UV/Vis- Spectrophotometry,
	Infrared Spectrometry
	Nuclear Magnetic Resonance Spectrometry
1970	Thin Layer Chromatography

	Gas Chromatography
	High Performance Liquid Chromatography
1980	Immunoassays'
	Gas Chromatography coupled to Mass Spectrometry
	Liquid Chromatography coupled to Mass Spectrometry
	Capillary electrophoresis
2000	Liquid Chromatography coupled to Tandem-Mass Spectrometry
	Liquid Chromatography coupled to Time-of-Flight-Mass Spectrometry
	Liquid Chromatography coupled to Chemiluminescence Nitrogen Detector
2005	LC/MS-MS Run Fraction Collection and subsequent Re-analysis by Chip-based Infusion on various Types of Mass Analyzers

Table 1. Timetable of Analytical Toxicology Techniques used for Organic Compounds

Years	**Analytical Technologies**
<1960	Inorganic Classical Analysis
	Emission Photometry
1960	Polarography
1970	Atomic Absorption Spectrometry
	X-ray Fluorescence Spectrometry
	Ion-Specific Electrodes
	Ion Chromatography
1980	Inductively coupled Plasma Mass Spectrometry

Table 2. Timetable of Analytical Toxicology Techniques used for Inorganic Compounds

For inorganic substances analysis of metals by atomic absorption spectrometry (AAS) was developed during the 1950s by Australian Chemist A. Walsh et al and further developed with electrothermal atomizers by Boris V. L'vov at Saint Petersburg Polytechnical Institute, Russia. More recently the hyphenated inductively coupled plasma mass spectrometry (ICP-MS) was pioneered by Scott D. Tanner and Vladimir I. Baranov from Perkin-Elmer Sciex Instruments. Inorganic anions detection and quantification by ion chromatography or ion exchange chromatography (IEC) was developed in the 1970s by Hamish Small and co-workers.

Evolution of Criminal Poisoning

From Antiquity to Renaissance it was very difficult if not impossible to prove poisoning by scientific evidence. Autopsies were only exceptionally performed. Proof of poisoning was made by gossip, circumstances, e.g. discoloration of the corpse, or poisons found at victim's neighborhood, sometimes animal testing, and by confession or by torture. As poisons were difficult to detect before 1800 and legislation inadequate to assist investigators in proving that criminal poisoning has been committed, homicidal poisoning was far more frequent than today. As progress of toxicological analysis in the 19th Century made detection increasingly more likely and as the sale of poisons became better controlled through strong legislation, the number of criminal poisoning cases decreased, despite the fact that enormously more poisons are available at present time. However poisoners have not disappeared today and victims remained the same over the years: unwanted family members or ex-lovers, rivals, employers or individuals killed to grift life-insurance money. Most cases concern family affairs, and in many cases health professionals are involved.

From the 15th to 17th Century schools for poisoners were established in Venice and Rome, known as the *"Italian School of Poisoners"* where some alchemists offered their *"elimination services"* as poisoners for fee. Many poison recipes were preserved in *"Secreta Secretissima"* archives, notably mercury salts such as $HgCl_2$, arsenic compounds such as As_2O_3, As_2S_3, or $AsCl_3$, *aconitum spp*, *taxus baccata*, bitter almonds, etc. Poisons were administered either as laced wines, in small pills or from a piece of jewellery called "poison ring". In the history of France some royal poisoners are suspected notably Charles the Bad, Louis XI, and Catherine de Médicis (1519-1589) married to King Henri II, alleged to have transferred the Italian knowledge on poisons to France, for the *"Parisian School of Poisoners"*. In the 17th century under Louis XIV (1638-1715) the so-called *"Affaire des Poisons"* occurred from 1670 to 1682, and generated many victims including children. Later in France between 1825 and 1900 some 70 criminal poisoning cases and from 1901 to 1975 only 10 cases have been reported. In recent past during 28 years some 32 homicidal poisoning cases with only one fatality have been admitted at the Parisian *Hôpital Fernand Vidal*, specialized in the medical treatment of poisoned patients. In the United Kingdom, poisoning had its heydays in the Victorian period (1819-1901) where some 504 criminal poisoning cases between 1750 and 1914 were reported including 237 arsenic cases. Many famous criminal poisoning cases were milestones in toxicology and contributed to its progress, even if "experts' wars" did not always increase the toxicologist's reputation by providing evidence at Court. Despite of this spectacular progress in forensic sciences, there are also some negative aspects partly due to the so-called CSI-effect. According to a FBI report 2008 the homicide clearance rate has decreased from 91 % to 63 % as criminals also became better informed and more precautious.

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Interesting Website on history of toxicology …http://toxipedia.org/display/toxipedia/Milestones+of+Toxicology

Biographical Sketch

Dr Robert WENNIG born 1942, studied chemistry in Luxembourg and Strasbourg where he obtained his PhD in 1970, was head of the forensic and clinical toxicology department at the Laboratoire National de Santé until December 2009 in Luxembourg. In 1986 he received his Forensic Toxicologist certification from the German society of forensic toxicology GTFCh. His academic career started as teaching assistant in 1965 at Strasbourg and Luxembourg in 1971, followed by a professorship in 1980 at Luxembourg University. He was in charge of GTFCh continuous education for many years and supervised post-graduate students at Strasbourg, Nancy and Metz Universities. His research topics were focused on pesticides, drugs-of-abuse, chronic toxicity biomarkers, and wild mushrooms. He served in several scientific societies: e.g as president of TIAFT; president of the Toxicological Society of Belgium and Luxembourg; vice-president of GTFCh; vice-president of the Medical Sciences Society in Luxembourg. He was a consultant to EMCDDA in Lisbon; expert at EMEA in London; an IPCS national correspondent

to WHO and his institute served as reference laboratory to UNDOC. In 2005 he received the AS Curry Award from TIAFT; in 2010 the Grand Prix de la Société Française de Toxicologie Analytique, and in 2011 the Jean-Servais-Stas Medal from the GTFCh. He is author or co-author of more than 200 scientific articles or book chapters and was a platform presenter of more than 400 topics.