10 Forensic Toxicology

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10.1 Introduction

The practice of forensic toxicology differs from that of clinical toxicology. The difference resides in the fact that suspicion and confirmation of intoxication must be supported by analytical assessment and not necessarily the response to treatment. The analytical investigation starts and ends with:

1. The heightened suspicion of intoxication based on clinical or post-mortem signs.
2. The appropriate identification of the toxin or class of the intoxicating agent.
3. The collection and handling of the appropriate samples to ensure accurate poison or toxin identification.
4. The selection of an appropriately certified forensic laboratory.
5. The documentation, chain of custody, of samples collected and submitted.

Toxicological diagnosis must also include the timeline associated with the biological steps of absorption, distribution, metabolism and, if known, excretion of the intoxicant.

Safety during the investigation of a possible intoxication must take priority for the investigator and their staff. Toxins may be viable at the crime scene and during the antemortem and post-mortem examination.

Finally, the forensic report should be inclusive of all findings, the reasonable suspicion (signs or symptoms) for the initiation of an investigation and the conclusions based on toxicokinetics, chemical analysis and the biological evidence.

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10.2 Forensic Toxicology Scope of Practice

Current criminal and civil investigations often rely on many specialized experts. Among these scientific experts is the Forensic Veterinarian. In particular, the veterinary forensic toxicologist provides an integral function in the exploration of illegal activity involving the death of animals. Forensic toxicology is the art and science of the identification of drugs (medically significant), poisons (chemical origin) or toxins (natural origin) that are of medico-legal interest. The goal set forth for the forensic toxicologist is to develop a narrative and timeline related to the events in question using their advanced education in investigative techniques coupled with an understanding of internal medicine, toxicology, pharmacology, biochemistry, chemistry, anatomical pathology, clinical pathology, physiology and anatomy.

The incidence of malicious poisonings is thought to be in the order of 1% of animal cruelty cases investigated in New York City (Wismer, 2014). This low incidence is likely to be due to a number of factors: failure to recognize the possibility of a poisoning event; failure to complete a thorough crime scene investigation; failure of veterinarians, law enforcement and animal control officers to report suspected poisonings; and the possibility that owners find their pet dead and dismiss the possible cause, disposing of the remains without investigation. The recognition of a toxic or poisoning event requires both observation and investigatory skill.

Suspicion of a poisoning event should be heightened by the following features: an otherwise healthy animal that dies acutely; residue or odour of a chemical nature on the coat, mouth, stomach or intestinal contents. Staining of the tongue, lips or peri-oral areas are also signs that should raise the level of suspicion of a toxicological event (see Table 10.1; Gwaltney-Brant, 2007).

The investigating veterinarian or agent must take precautions during the investigation. The ante-mortem signs of intoxication vary with the poison. The post-mortem examination of the remains must be carried out with extreme caution, as some toxins (e.g. zinc phosphide) have been known to affect the individuals in contact with the remains (Guidechem, 2006; Papenfuss, 2012). Many dangerous chemicals have been restricted in their availability to the general public; however, some quantities of restricted or prohibited poisons and pesticides may be available from stored stockpiles and be accessed by ranchers, farmers and agriculturalists. The forensic veterinarian is well advised to perform all necropsies in well-ventilated areas with the appropriate protective equipment to avoid self-contamination, injury or even death.

The forensic toxicologist should always be aware of possible confounding evidence that could be misinterpreted as a malicious poisoning (e.g. carbon monoxide death after a fire event, sudden livestock deaths after exposure to local toxic plant). In each of these cases a complete crime scene investigation and necropsy should assist in the differentiation of malicious and accidental intoxications (Smith, 1996).

Forensic veterinarians differ in their application of toxicological principles from the veterinary clinical toxicologist. The forensic practitioner uses documentation to record significant events during the investigation. The permanent written record of the collection, maintenance and storage of evidence is also termed the chain of custody. The chain of custody includes all findings related to the initial scene of activity, the patient or cadaver and every aspect of the samples of evidentiary value collected (e.g. the time, location, the person collecting the samples or tissues, methods of storage or transport and the identity of all individuals handling the samples). In the ante-mortem patient, the condition of the patient (signs and symptoms), the timing of treatments and the collection of forensic samples (e.g. blood, urine or biopsy tissue) must also be noted. This documentation is maintained for all samples and events from the crime scene through the final judicial process.

10.3 Sample Collection

Obtaining appropriate samples involves an understanding of: the nature of the investigation (e.g. questions being asked by lead investigators); the crime scene; the species

Table 10.1. Examples of common poisons of forensic concern (Gwaltney-Brant, 2007; Wismer, 2014).

Poison	Exposure	Chemical State	Mode of Action	Ante-mortem Signs	Post-mortem Signs	Toxic Dose (LD50)
Ethylene Glycol	Oral	Liquid	Oxalic acid crystal and renal tubule blockage	Vomiting, ataxia, anorexia hypocalcaemia	Birefringent crystals in kidney	Cat 2–4 mg/kg Dog 4–5 mg/kg
Rodenticide -Strychnine	Oral	Powder or pellet	Block neuro-inhibitory transmission	Stiffness, tense abdomen Tetanic seizures started by startle	Rapid rigor Cyanosis Petechial/ecchymotic haemorrhage	Bovine 0.5 mg/kg Equine 0.5 mg/kg Canine 0.75 mg/kg Feline 2.0 mg/kg
Rodenticide -Anticoagulant -Warfarin -Dicumerol -Diphacinone, etc.	Oral	Pellet	Block Vitamin K-dependent coagulation	Skin haemorrhage: -ecchymotic -petechial weakness Anaemia Bloody froth mouth and nose	Haemorrhages: -pulmonary -intraocular -subdural -cerebral, etc. Free blood in body cavities Heart flaccid	Dog 50 mg/kg Cat 25–50 mg/kg
Rodenticide -Metal-Phosphine -Zinc phosphide, etc.	Oral	Powder or pellet	Phosphine gas blocks mitochondrial respiration, protein and enzyme synthesis	Convulsions Vomiting Dyspnoea Weakness Sudden death	Congestion -lungs -kidney (tubular degeneration) Liver (yellow mottling)	Dog 300 mg/kg Very toxic to human investigators[a]
Rodenticide -Bromethalin, etc.	Oral	Pale crystalline powder	Uncouple cellular oxidative phosphorylation	Ataxia Extensor rigidity Clonic seizures Respiratory arrest Muscle Fasciculation Hyperexcitability	CNS Spongy demyelination Cerebral oedema Inflammation of: -liver -kidney -Bowman's capsule	Cat 1.8 mg/kg Dog 4.2 mg/kg
Rodenticide -Calciferol, etc.	Oral	Powder	Increase circulating calcium	Cardiac arrhythmia Depression Inappetance Polyuria Polydipsia Increase blood pressure	Mineralization -blood vessels -kidney -stomach -lungs	Cat - low toxicity Dog 4.7 mg/ml
Dipyridyl herbicide -Paraquat	Oral Skin	Liquid Pellets	Pulmonary free radicals	Vomiting Depression Acute dyspnea Cyanosis	Alveolar fibrosis Atelectisis Hemorrhagic -intra alveolar	Dog 25–30 mg/kg Cat 40 mg/kg

of animal involved; ante-mortem signs and symptoms; and a review of the investigation documentation (police reports, witness reports, crime scene photography) (Young and Ortmeier, 2011). Further, the forensic practitioner should anticipate the questions that could reasonably be asked in the future, including in the court and depositions.

In ante-mortem toxicology, the practitioner should conduct a physical examination (including laboratory evaluation) of all live animals before continuing to the post-mortem patient(s). In the case of the ante-mortem examination (hair, fur, blood and urine) samples should be collected before medical treatment is initiated. Similarly, in the investigation for performance-enhancing drugs, samples should be obtained as soon as the animal athlete is finished with the competition. Any delay after the completion of the competition may taint results or break the chain of custody (Gwaltney-Brant, 2007).

Questions that surround a post-mortem examination may involve the effects of a chemical substance either found at the scene or in the possession of a person of interest. Due to the labile nature of many toxins, drugs or poisons, the accuracy of an analysis depends on a number of factors: time since ingestion or administration; the nature of the substance of interest; ambient weather conditions; amount of decomposition and location of the remains (buried above ground versus below ground versus in water). Investigation of the primary crime scene may reveal evidence of vomitus, salivation, lacrimation, significant toxic flora, chemical staining, unusual odours or residue on tissues or infestation by insects (Gwaltney-Brant, 2007; Rao, 2012). In post-mortem cases, without ante-mortem signs the diagnosis of the toxicological cause of death can be very challenging and solely dependent on the necropsy and the analytical evaluation of entomological samples (AFMES, 2012). Sufficient samples should be obtained and stored in order to ensure access to testing by other appropriate parties (defence attorney, governmental agencies, etc.) and to repeat and confirm initial results (see Table 10.2).

When possible, the direct collection of evidence from the crime scene (fur, faeces, vomitus, suspected debris, chemical residue, and used or discarded containers) is optimal. In the case of a post-mortem investigation, samples include all body tissues and fluid samples that are available.

Hair and fur should be shaved down to allow examination of the skin for signs of parenteral drug or toxin administration. All body orifices must be examined for evidence of drug or toxin placement. Radiographs may reveal unusual substances, foreign bodies or other evidence in the gastrointestinal tract or body cavity. Though not well established in animal forensic investigations, the fur/hair sample may be used as circumstantial evidence to establish identity between an unknown fur source and an exemplar of fur from a suspect animal. Some initial research has been completed in hair or fur analysis for cortisol levels over time (Bryan *et al.*, 2013). Human hair analysis for illicit drugs has been a forensic investigatory technique to establish and monitor illegal drug use (Ledgerwood *et al.*, 2008). The future of these techniques to analyse and match drug concentrations in animal hair may be another method of comparison and identification of a known and unknown sample.

The submission of specimens should be accompanied by suggestions of the suspected class of poison, toxin or drug. The chemical class may be suspected from: ante-mortem signs and symptoms (from clinical examination, review of witness reports, etc.); necropsy findings; crime scene evidence; and in some cases following a court warranted search of suspect premises. Without guidance as to the nature of the toxin/poison, many laboratories may be unable to complete a forensic analysis of the samples submitted.

False positives and false negatives may be obtained due to unexpected chemicals, poor evidence collection or contamination. To ensure accurate and appropriate test results, the forensic scientist and the forensic analyst must be aware of test limitations and the possible existence of interfering agents. Many of the issues of contamination are addressed by maintaining strict chain of custody protocol and paperwork from the crime scene to the laboratory. Not all analytical laboratories can maintain the chain of custody. Samples are best submitted either by commercial courier or other traceable delivery system. Transport by a member of the

Table 10.2. Appropriate toxicological samples collected during ante-mortem and post-mortem examinations (Cooper and Cooper, 2007; AFMES, 2012).

Ante-mortem Samples	Collection Transport	Post-mortem Samples (fluid)	Collection Transport	Post-mortem Samples (tissue)	Collection Transport
Urine	Fresh sample Glass/plastic vial	Vitreous humour	All, 2%Na fluoride	Liver (right lobe deep)	50–100 g unfixed, plastic or glass
Venous blood[a]	15 ml Na fluoride tube 10 ml K EDTA tube Extra aliquots (50 ml)	Stomach contents	Fresh, unfixed plastic or glass	Brain	100–200 g, fresh, fixed in plastic or glass
Hair/fur	100–200 mg Envelope or aluminium foil[b]	Blood[a] -cardiac (rt. atrium) -inferior vena cava	30 ml Na fluoride 30 ml K oxalate Extra aliquot (50 ml)	Subcutaneous fat	100–200 g, glass or plastic
Vomitus	All collected Glass vial	Urine	Fresh sample	Kidney	25–50 g, glass or plastic
Faeces	All collected Glass vial	Bile	All, fresh sample	Skeletal muscle Psoas, deep thigh, spinal muscle	100 g, fresh in glass or plastic
		Cerebral spinal fluid	All, glass or plastic	Lung	Apex tissue 25–50 g, glass or plastic
				Hair/fur	100–200 mg Envelope or aluminium foil[b]

[a]Avoid serum separation tubes as the gel may absorb drugs or toxins.
[b]May use glassine or paper.

forensic team or police force directly to the laboratory is also acceptable. When submitting toxin, drug or poison samples for chemical evaluation, the practitioner must be aware of the receiving laboratory limitations and strengths. In many cases, failing to follow the specific sample submission protocols may result in compromising both the chain of custody and the overall criminal or civil investigation.

Often, the biological remains are infested by insects after death. Entomology measures the assessment of the time since death and is well-established science based on the presence and growth of various insect species, including assessment of the stages of the Green Bottle fly (order Diptera) (Amendt *et al.*, 2007; Cooper and Cooper, 2007). Where degradation is advanced, the collection of arthropod samples for entomotoxicology is an alternative to direct tissue/body fluid analysis. Entomotoxicology is the established science of assessing the effects of common drugs on the growth rate and activity of arthropod species in decomposing animals. This technique allows the investigator another tool to assess the presence of drugs in the remains, weeks or months after death (Beyer *et al.*, 1980; Gagliano-Candela and Avetaggiato, 2001). In human forensic sciences, controversy exists among analysts who disagree over the usefulness of the analysis of maggots, since there appears to be little correlation between chemical analysis of arthropods and the quantification of drug in the cadaver (Tracqui *et al.*, 2004). However, unlike our forensic physician counterparts, the establishment of any quantity of illegal drug in an animal cadaver is cause for suspicion of abuse. To appropriately submit samples for entomotoxicology, the forensic veterinarian should visit the crime scene and confirm the presence of arthropods on the remains: in particular the

larva of Diptera and the beetles (order Coleoptera), which feed directly on the larva (Gagliano-Candela and Avetaggiato, 2001). Collected arthropods should be sampled while on, or in, the body as opposed to those found in the environment surrounding the remains.

10.4 Animal Athletes and Performance-enhancing Drugs

The forensic veterinarian may be called to ascertain whether an animal athlete is using performance-altering drugs. This type of investigation follows the human concerns of athlete doping and chemical enhancement of performance. The World Anti-Doping Agency (WADA) concerns itself with the evaluation of the human athlete performance by monitoring the use and abuse of drugs altering human physiology.

The animal athlete may also be chemically altered to enhance performance, reduce signs of over-exertion and mask injury. The use of performance-enhancing drugs in horse racing and dog racing has resulted in tragic injury following the catastrophic failure of the animal in full race mode, resulting in both animal and human injury (Huntington, 2011; Animal House, 2013; Richardson, 2013).

Monitoring of performance drugs is limited to animal sports regulatory agencies, animal cruelty monitoring agencies (e.g. People for the Ethical Treatment of Animals), and overseeing bodies for the horse and dog racing industries. The veterinarians used to monitor and collect appropriate samples from those animals in the top tiers of a race, are often under the control and regulations of the state or federal governmental agricultural or gaming agencies. Though not a direct responsibility of law enforcement, the forensic veterinarian may still have need to investigate the illicit or illegal use of performance-enhancing drugs in animals.

There are efforts under way in the USA to ban illegal drugs in the horse racing industry so as to reduce the risk of death and injury to animal and jockey. Currently in the USA there are no national testing systems in place (Butler, 2010). Further, some state legislatures have proposed that an organization similar to WADA be established. In the north-east USA there is a governmental bill to establish an enforcement organization for the regulation of the prohibition of animal doping (e.g. Horseracing Integrity and Safety Act of 2013). This trend of calling for increased oversight and enforcement of animal doping is likely to expand across North America (Racing Medication and Testing Consortium, Kentucky, USA), and Europe (Fédération Equestre Internationale, Lausanne, Switzerland).

In many cases, where the forensic veterinarian is alerted and activated to investigate a case of animal doping, there are criminal or civil issues beyond the importance of the successful completion of an athletic event. Many insurance claims, wrongful death litigation and events with consequential human injury may be tied to animal performance drugs. The forensic veterinary toxicologist should have some familiarity with the illegal use of performance drugs in animal sports (Table 10.3).

In some cases the forensic toxicologist may be asked to evaluate performance-enhancing drugs related to reproduction, growth or bio-production (e.g. milk). Forensic investigations may also require the investigation of the use of antibiotics in food animals (European Union) or restricted antibiotics (North America and Asia). In many cases, the use of restricted pharmaceuticals in food-producing animals is monitored by the department of agriculture for the country or region involved. The forensic toxicologist when activated to investigate these occurrences should use the appropriate agricultural or food animal testing laboratory to ensure accurate results.

10.5 Selection of a Forensic Laboratory

The selection of an appropriate laboratory for toxicological analysis is intimately tied to the legal acceptance of the expert analysis and the testimony of a forensic veterinary toxicologist, and establishes the nature of the challenges from the opposing attorney or their expert witnesses.

Historically, in the USA, the accreditation of laboratories is required to ensure the highest standards of analytical science and toxicology. Two court decisions in the USA resulted in

Table 10.3. Performance-enhancing drugs in animal athletes (Butler, 2010; Huntington, 2011; Richardson, 2013).

Drug	Species	Use	Bio-samples	Detection Method	Comments
Sildenafil (Viagra)	Equine	Vasodilatation Increase lung perfusion	Plasma, serum, whole blood	Chromatography Mass spectrometry	
Anabolic Steroids (Stanozolol, etc.)	Equine, Canine	Increase muscle mass and endurance	Urine	ELISA	
Corticosteroids	Equine, Canine	Decrease inflammation Analgesia	Urine	ELISA	
Sodium bicarbonate (Milk Shake)	Equine	Decrease lactic acidosis Decrease fatigue	Blood	Plasma total carbon dioxide (TCO2)	Confound: commercial forage/grain
Arsenic	Equine	Low dose stimulant Higher dose decrease performance	Blood, hair, urine, soft tissue	Multiple tests: Marsh Test Atomic absorption Neutron activation	
Dimethyl sulfoxide (DMSO)	Equine	Analgesia Decrease inflammation	Urine	Chromatography Mass spectrometry	Confound lucerne grass
Methyl xanthenes (caffeine)	Equine	Stimulant Bronchodilator Vasodilator	Urine	ELISA	

setting the quality standards and expectations for use of science in the court system. The Frye Standard[i] and the Daubert Standard[ii] were the basis for challenging 'junk science' testimony in the court. Combined with other rulings, these decisions resulted in the establishment of the US Federal Rules of Evidence guiding the use of science techniques and testimony in a medico-legal forum. Science must be based on well-founded research when used to establish or refute criminal guilt, innocence or responsibility, in a civil hearing. The Federal Rules of evidence set forth four standards for the acceptance of good science in the court:

1. The scientific technique or method must be tested and considered valid by the educated scientific community.

2. The error rate for any technique (e.g. false positives versus false negatives) must be known and within acceptable scientific limits.

3. The techniques used must have been reviewed and be acceptable in peer-reviewed journals for their accuracy, specificity, selectivity and repeatability.

4. The testing and the equipment used must be generally accepted by the scientific community as appropriate for the test completed.[1]

Though these rules are only binding on the acceptance of scientific evidence and expert testimony presented before the US Supreme Court, many other courts have accepted this guidance for the evaluation of the quality and validity of scientific evidence. To this goal, forensic laboratories in general, and forensic toxicology laboratories in particular, should meet all regulatory standards for techniques, equipment, staff, training and facilities. Only those laboratories compliant with the regional standards, with appropriate certifications and qualified staff, should be engaged by the veterinary forensic toxicologist to ensure acceptance of the expert scientific and toxicological conclusions presented in the legal forum.

[i] *Frye vs United States* (1923) 293 F.1013, D.C. Circuit Court. Available online at: http://www.law.ufl.edu/_pdf/faculty/little/topic8.pdf (accessed 25 August 2014).

[ii] *Daubert vs Merrill Dow Pharmaceuticals Inc.* (1993) 509 US Supreme Court 579. Available online at: https://www.law.cornell.edu/supct/html/92-102.ZS.html (accessed 25 August 2014).

Specifics of the laboratory-accreditation procedures and processes vary among the multiple regulatory organizations and countries. In general, laboratory accreditation indicates that there is regular external oversight and monitoring of forensic laboratory procedures, techniques, calibration of equipment, and continuing education standards for technical and supervisory staff. Quality control programmes must be in place to ensure accurate results. There is proficiency testing of technical staff using unknown samples interspersed with regular forensic samples.

Internationally, the establishment of forensic and crime laboratory standards involves several nations in North America, Europe and Asia. The International Organization for Standardization (ISO) has set laboratory accreditation standards, ISO 17025. These are standards for performing tests and equipment calibration. In the European Union (EU), the forensic laboratory accrediting body is the International Laboratory Accreditation Cooperation (ILAC). The European Network of Forensic Science Institutes (ENFSI) also oversees laboratories in more than 20 EU nations. The combination of oversight and regulatory standards issued by ISO, ILAC and ENFSI support forensic laboratory accreditation for the majority of the crime laboratories outside the US and Canada. ISO standards are recognized by forensic laboratories in North America (Whatwhen-how.com, 2010).

In North America forensic laboratories follow several standards to be qualified for forensic investigation analytical work. The College of American Pathologists (CAP) issues written standards for evaluation of the proficiency of laboratory testing. CAP has several areas of laboratory accreditation for human testing. The accreditations that are ensuring accuracy for forensic testing include:

1. Laboratory Accreditation Program.
2. Forensic Urine Drug Testing.
3. Athletic Drug Testing Program.

The American Society of Crime Laboratory Directors (ASCLD) offers a laboratory certification programme. Finally the American Board of Forensic Toxicology Inc. (ABFT) is another certification accepted by the court as ensuring quality testing and standards (Whatwhen-how.com, 2010). To date, the laboratory certification programs are acknowledged to monitor primarily human-testing laboratories. The excellent standards set by these certifications allows confidence in the toxicological results for animal samples, and acceptability by the court and legal system.

10.6 Methods of Toxicological Analyses

In the selection of an analytical testing laboratory, several criteria should be considered. The nature and robustness of any forensic testing procedure depends on its accuracy, precision, specificity, selectivity and sensitivity. Technical analytical procedures depend on a number of factors:

1. Type and quality of the samples submitted (fluid versus tissue).
2. Availability of analytical techniques and equipment.
3. Expertise of the laboratory and analytical toxicologist.
4. Nature of the suspected toxin (drug or poison).
5. Complexity of the specimen preparation for analysis (the more complex the procedure the more likely the defence will dispute the results).
6. The legal and scientific acceptability of the method to be used.

The molecular nature of toxicants suspected will determine the requirements for the upper and lower limits of detection (LOD). The selection of the signal must be significantly greater than the background noise to ensure an acceptable signal to noise ratio (S/N) and therefore acceptance in method validation and hence in a court of law (Peters and Maurer, 2002). Often the presence of a poison in a body is sufficient to confirm criminal activity (e.g. strychnine, cyanine). Quantification of a drug, toxin or poison may be necessary in the case where there is interference from metabolic products or feeds. Decisions made for the analysis of forensic evidence should be completed with the assistance of an experienced laboratory toxicologist.

There are both screening tests and confirmatory tests. Screening tests can be used to differentiate a presumptive positive test for a drug from a negative. There should always be sufficient biological samples so that screening tests may be followed by a confirmatory test. The confirmatory test is used to definitively identify the toxin, poison or drug and to avoid false positives or false negatives (Dolinak, 2005; What-when-how.com, 2010; Caplan and Kwong, 2012).

Screening tests may be used either at the crime scene or in the clinical setting. This is a common method of analysis for the drugs of abuse or performance-enhancing drugs (Caplan and Kwong, 2012). Obtaining immediate samples of urine and blood may allow the use of field drug tests to identify the drug(s) in the system. Tests are available through law enforcement and forensic suppliers. Many types of urine/drug analysis kits are available as over-the-counter products at local pharmacies. This information must not be relied upon to the exclusion of other possible toxins or poisons. Often a confirmatory test is supportive to the diagnosis.

Confirmatory tests are performed in a laboratory setting. These tests occur in two stages, beginning with the separation of the suspected chemical analyte from the biomaterial submitted, and then the detection of the chemically unique characteristics of the analyte for identification. Separation methods include enzyme-linked immunoassay (ELISA), chromatography and capillary electrophoresis (CE). Once separated, the chemical analyte is detected based on a unique molecular or chemical characteristic for a definitive identification. Selection of the method of separation and detection may be limited either by standards against which the unknown is compared or due to a limited library of detector results available to identify the unknown (Caplan and Kwong, 2012).

ELISA is often used for the identification of drugs (performance-enhancing, abuse) or known chemical substances of abuse. The method involves using the unknown sample antigen (analyte) attached to the surface of a non-mobile phase or well. The known detection antibody is intimately linked to an enzyme and allowed to interact with the antigen. Multiple liquid agents are then added to the analyte. The analyte is washed and eventually leads to a colour change, concluding with the identification of the sample (Gomolka, 2012). The ELISA tests are limited by the availability of known standard detection antibodies. Although many enzyme-linked antibodies are available, the development of additional testing antibodies is often an expensive and time-consuming task. Another separation method, and one that is more widely used as a laboratory method, is that of chromatography coupled with a mass spectrometer.

Chromatography separates an unknown chemical entity for analysis from biological material. Chromatography methods vary, based on their use of either a liquid or a gas mobile phase for separation. These techniques include liquid chromatography (LC), gas chromatography (GC) and high-performance/pressure liquid chromatography (HPLC). Once separated, the components pass through a detector that is continuous with the chromatography unit. The unique properties of the analyte are then detected and changed to an identifiable signal (e.g. electrical or photometric). The detector output is translated to a data format stored either in a computer interface or as a printed paper record. The selection of a separation method and detector used is critical to the medico-legal validity and conclusions drawn by the forensic scientist.

The most commonly used detector is the mass spectrometer (MS). The MS identifies and quantifies drug of abuse and illicit pharmaceuticals based on their unique chemical characteristics (Cody, 2003). For the MS there are known libraries identifying specific known compounds. The standards contained in the known library are compared to the unknown chemical analyte. This results in the identification and confirmation of the unknown sample. Several detectors are modifications of a basic MS detector. These include quadrapole MS (QMS), ion trap MS (ITMS), time of flight MS (TOFMS), and Fourier transform ion cyclotron resonance MS (FTMS) (Stafford *et al.*, 1984; Ojanpera *et al.*, 2005). The various

MS techniques differ in cost and ease of operation, LOD and availability of known standards. Choice will be dictated by the nature of the sample analyte presented or suspected. Currently the gold standard for forensic analysis is HPLC-MS. The use of the HPLC-MS (FTMS) is considered of superior forensic value because of its greater sensitivity, specificity and accuracy compared to other chromatographic mass-spectrometer techniques (Valaskovic *et al.*, 1996). Currently, HPLC-MS (FTMS) is of limited use in forensics due to the extreme high cost of the equipment and ultra-low vacuum requirements for use (Smith *et al.*, 2007).

Capillary electrophoresis (CE) is of increasing use in forensic toxicology laboratories due to its relatively low cost, and its ease of set-up and use. This separation method uses either a liquid or solid medium phase for separating chemical components based on electrophorectic direction and mobility (differentiation based on molecular positive and negative electrical charges). Detectors available for use with CE include ultraviolet light, visible light, florescence spectrophotometers, mass spectrometers and pulsed electrochemical detectors (Smith *et al.*, 2007).

10.7 Principles of Toxicokinetics

Toxicokinetics and toxicodynamics refer to the nature of the distribution of toxins, poisons or drugs through the body from absorption to excretion. In many cases, the method of absorption can determine the toxicity of a chemical. The method of exposure to an agent may be determined by a close external and internal examination of the victim for oral, rectal or parenteral administrations (both ante-mortem and post-mortem). The rate of absorption is critical, as some toxins, if absorbed slowly, may be significantly less toxic than when rapidly absorbed. The chemical nature of the toxin, poison or drug may allow for the establishment of a timeline prior to death. The rate and method of absorption, target organs, mode of action, mode of metabolism and excretion all play critical roles in determining the motivation of an intoxication. This is a significant medico-legal issue in the consideration of criminal or civil culpability (Dolinak and Matshes, 2005).

Exposure to a lethal substance often results in variations in the toxic and lethal response of individuals to the same dose and toxin. The toxin in a group of animals results in some percentage of morbidity versus mortality. The toxic effect of a chemical is described as the lethal dose resulting in death of 50% of animals exposed (LD50). Variability of the lethal effects of a toxin, poison or drug is based on multiple factors. Toxicity of a chemical is described by its concentrations in target organ(s) (Rao, 2012).

The forensic toxicologist must be cognizant of all of the factors that may affect the lethality of a chemical entity. This describes the risk assessment for the exposed animals. Biological variability of individuals and among species is based on differences in genetics, physiology and biochemical metabolism. Other factors that act to enhance variability include type of exposure (acute versus chronic, route), and age, health and reproductive status of the animals exposed. Finally the environmental conditions may have a synergistic or antagonistic action on the chemical lethality. Considering the physiological and genetic factors of the organism, and the influence of the molecular chemistry of the toxicant on tissue concentrations (and therefore its lethality) may be defined as toxicokinetics. It is the science of toxicokinetics that determines the rationale for the tissue and fluid sampling of the previous sections (Poklis, 1996).

Toxins may enter the body either through dermal, inhalation, ingestion or injection routes. The absorption characteristics of each of these methods of exposure are very different and contribute to the eventual mortality of the animal. Bioavailability is also dependent on the chemical phase and molecular structure of the toxicant. Tissue levels will rise very rapidly with inhalation, ingestion or injection, while dermal exposures are often more slowly absorbed. The rapid rise in toxin concentrations at the target organs for the toxin or poison may be the difference in an acute versus a chronic lethal event (Dolinak and Matshes, 2005).

Once absorbed, the poison will be distributed via the vascular or lymphatic systems, diffusion or active transport, facilitated passive transport or pinocytosis. Distribution is not the same to all organs and tissues due to different blood perfusion percentages, varying lipid content, tissue pH and cellular activity or metabolism. The distribution of a toxicant in the body is described as the volume of distribution (Vd, reported as milligrams of toxicant/millilitres of blood volume). Rarely is the dose of a toxicant known at the time of the investigation; however, the identification of the toxin or poison and knowledge of the volume of distribution for a given chemical entity will help the forensic toxicologist understand the concentrations of the drug in the individual tissues sampled and analysed (Rozman and Klassen, 1996; Rao, 2012).

The metabolism of a toxin or poison may either decrease toxicity or enhance toxicity, dependent on the nature of the metabolites formed. Metabolism can involve the liver, lungs or kidney. In many cases, the original chemical entity will be metabolized to increase its ability to be excreted from the body. The rate of metabolism varies between individuals. Once formed, the metabolites will be excreted by the kidney in the urine, by the liver in the bile or by the lungs as exhaled gas (Rozman and Klassen, 1996; Rao, 2012).

Excretion of the metabolic products results in decreasing toxicity as they are removed from the body. In some cases the decreasing toxicity may result in morbidity without mortality. In cases where the metabolites have a higher toxicity than the parent compound, mortality may be delayed as the metabolites concentrate in the body system. The distribution and metabolism of a toxicant must be considered when examining the antemortem patient (Dolinak, 2005).

10.8 Conclusions

Veterinary forensic toxicology involves the scientific assessment of animal intoxications by various chemical, pharmaceutical or toxic agents. The role of the investigator is to gather all the pertinent data relating to the suspected crime from the crime scene. This evidence must then be catalogued, recorded and secured. The conclusions drawn from the necropsy, along with reviewed police reports, medical records and witness reports, allows the veterinarian to establish a level of suspicion as to the possibility of a toxic event. There may even be sufficient evidence to give an indication as to the class of poison/toxin or a specific poison. The forensic investigator may then submit the body tissue and fluid samples to an analytical laboratory. The forensic investigator is responsible for selecting an appropriately qualified laboratory for the toxicological analysis. Once received, the investigator must assess the laboratory analytical report for validity and accuracy based on all the evidence gathered.

The veterinary forensic investigator plays a pivotal role in establishing the narrative of the sequence of events leading to the suspected crime. The narrative should encompass the biological, pathological and toxicological evidence that has allowed an informed opinion to be reached regarding the possible nature of death of the animal. A reconstruction of the timeline of events immediately preceding the death is the ultimate goal of these investigations.

The diversity of veterinary patients requires that toxicological conclusions be supported by the most current and accepted scientific and medical documentation. Therefore, veterinarians involved in the investigations of crimes against, or involving, animals should be cautious of assigning blame. Forensic scientists are not concerned with justice or injustice, guilt or innocence; they are only interested in the use of the best medical science and investigatory procedures to establish, support or disprove other evidence collected. In an effort to continually improve its art and science, veterinary forensic toxicology is currently utilizing the most modern scientific and investigative techniques.

This chapter has reviewed the salient factors that may be considered during a forensic investigation involving toxins or poisons. As crimes become more sophisticated, so must our observations, and levels of suspicion must become more acute. Technological

equipment and chemical analysis allows the toxicologist the ability to detect smaller quantities of toxins or poison that can result in morbidity or mortality. For each forensic investigator, working within the criminal legal system, there must be considerations of the cost/benefit of each test requested. Multiple testing without cause or scientific direction is to be avoided.

In conclusion, the critical link in the process of a toxicological investigation is the ability of the investigator. The astute perception and understanding of the elements of intoxication is the key to a successful conviction. The forensic toxicologist is responsible for the appropriate documentation and presentation of the evidence and conclusions.

Note

[1] Committee on the Judiciary, 112th Congress (2012) Federal Rules of Evidence 2013, in Federal Evidence Review. Available online at: http://federalevidence.com/downloads/rules.of.evidence.pdf (accessed 1 August 2014).

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